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THE INFLUENCE OF THYROID FEEDING UPON CARBOHYDRATE METABOLISM

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The relation of the thyroid gland to carbohydrate metabolism has been discussed from various view points. In Basedow's disease spontaneous glycosuria or alimentary glycosuria has been frequently recorded. Flesch (1) found alimentary hyperglycemia in 61 per cent of his cases of Basedow's disease and also was able to cause alimentary hyperglycemia by thyroid feeding or transplantation of the thyroid gland. Schulze (2) reported that alimentary glycosuria is not so frequent in Basdeow's disease (25 per cent), but a very small amount of epinephrine, which was insufficient to cause glycosuria in the normal condition, called forth marked glycosuria in 80 per cent of the cases of Basedow's disease examined. The tolerance for carbohydrate is considered to be increased in myxoedema. In cases of myxoedema and obesity, glycosuria was found after the use of thyroid preparations.

Hirsch (3) and Underhill and Saiki (4) demonstrated a decrease in the utilization of dextrose given by mouth or subcutaneously introduced in dogs after thyreoparathyroidectomy. Hirsch (5) showed later that although thyreoparathyroidectomy leads to a lowering of the tolerance for carbohydrate, thyroidectomy with undisturbed parathyroids produces no such effect. Demonstrating that thyroidectomy with preservation of the parathyroids increases the tolerance for sugar and inhibits epinephrine glycosuria, Eppinger, Falta and Rudinger (6), (7) came to the conclusion that the influence of parathyroidectomy upon carbohydrate metabolism is just contrary to that of thyroidectomy. As to the influence of thyroidectomy with preservation of the parathyroid upon epinephrine glycosuria, Underhill and Hilditch (8) could not confirm the results of Eppinger and his coworkers. Performing the same operation, Pick and Pineless (9) proved its inhibitory influence upon epinephrine glycosuria in young goats, but this was not the case in rabbits. McCurdy (10) observed an increased tolerance for dextrose injected intravenously in dogs from which the thyroid together with two parathyroids had been removed. In thyreoparathyroidectomized cats, Miura (11) demonstrated that this operation does not show any influence upon alimentary galactosuria, but it decreases epinephrine glycosuria. From the studies of

Eppinger, Falta and many others, it was shown that thyroid, chrômaffin tissue and hypophysis act in an accelerative manner upon metabolism whereas pancreas and parathyroid behave in the opposite way. The relation of various ductless glands must, therefore, be considered in discussing the influence of the thyroid gland upon carbohydrate metabolism.

When thyroid preparations are introduced into animals many pathological symptoms are called forth. Glycosuria is sometimes seen. Carlson and his coworkers (12) found that desiccated thyroid given by mouth is toxic for many animals and different genera exhibit great variation in their susceptibility, dogs, cats, foxes and ducks being the most resistant of all animals fed, rodents and primates the least. The most constant symptoms were emaciation and diarrhoea with death in convulsions or depression. French (13) also confirmed the toxicity of thyroid for rabbits, rats and guinea pigs, control experiments with other organs or tissues showing no toxic symptoms. When fresh thyroid was given by mouth 20 to 50 grams per day, the rabbits died in two to ten days. Giving fresh thyroid to rats and cats by mouth, Cramer and Krause (14) found that the glycogen content of the liver decreases to a minimum. In dog experiments they showed also that thyroid feeding causes a slight but distinct lowering of the tolerance for glucose. They concluded that thyroid, when administered to normal animals, acts specifically on only one aspect of carbohydrate metabolism; it inhibits the formation and storage of glycogen in the liver. Finding a change of the curve of the respiratory quotient in experimental hyperthyroidism, Cramer and McCall (15) concluded that thyroid hormone discharges and oxidizes the liver glycogen and this explains the increased oxidation of carbohydrate, protein and fat, produced by thyroid feeding.

Mobilization of liver glycogen can be induced by many factors, namely excessive muscle work, fasting, epinephrine injection, pancreas extirpation, piqûre, irritation of splanchnic nerves, intoxication with narcotics or phosphorus, etc. Though it is a well known fact that epinephrine injection discharges liver glycogen, Schwarz (16) made an interesting contribution which shows that extirpation of both adrenal glands also induces the total loss of liver glycogen in rats. On the other hand, Pollak (17) proved that rabbits, made glycogen-free by fasting and strychnine, show an increased glycogen content of the liver, in a quantity only equaled by carbohydrate-fed animals, after injections of small increasing doses of epinephrine. Kahn and Starkenstein (18) confirmed Schwarz's results in rat experiments, but in rabbit experiments they could not demonstrate any such influence. Investigating the relation between the thyroid and adrenal glands, Cramer (19) considers that the thyroid hormone stimulates the secretion of epinephrine and this produces a discharge of glycogen from the liver.

From the brief references mentioned above it will be seen that the problem of the influence of the thyroid gland upon carbohydrate metabolism is very complicated. At the suggestion of Prof. Frank P. Underhill, I have investigated a few points concerning this problem, namely the influence of thyroid feeding upon the glycogen content of the liver, blood sugar content, assimilation limit for dextrose and also upon epinephrine hyperglycemia and glycosuria.

METHODS

Rat experiments. Full-grown white rats were fed on paste, prepared according to Osborne and Mendel with 70 per cent of rat biscuits and 30 per cent of lard. Fresh thyroid glands of pigs obtained from the slaughter house or desiccated thyroid (Parke, Davis and Company) was added to the diet. In case of *fresh* thyroid glands, the amount for each animal per day was measured at the beginning of the experiment and kept in a freezing room. For control animals lamb meat was prepared in the same manner. Though the influence of the parathyroidectomy upon carbohydrate metabolism has become clear, the result of feeding this organ is yet unknown. The thyroid preparations used in these experiments may have contained a very small amount of parathyroid. The rat eats thyroid very willingly. When *desiccated* thyroid was used, it was mixed with the paste in the proportion of 1:9. For control experiments comminuted, desiccated, coagulated egg was mixed with the paste.

Before the real experimental period, all the animals were fed on paste regularly for at least three days. The urine was collected, following the method suggested by Osborne and Mendel (20). The rat does not eat much in the day time, and it is very difficult to compel it to eat a certain amount of food in a desired time. In my present work, thyroid or meat was given at 6 p.m. After the animal ate this food, the paste was given and left during the night. The next morning, the rest of the paste was taken away and no food was given in the day time. Water was given freely. On the last day, thyroid or meat was allowed as usual, but the paste was put in the cage at 10 p.m. At 9 the next morning the animal was killed. When thyroid was given the appetite of the animal became poor. The amount of food for each control animal was regulated so that it was about equal to that of the thyroid-fed animal.

Glycogen was determined by Pflüger's method (21), (22). The glycogen was hydrolyzed and the reducing sugar obtained was estimated by Allihn's gravimetric method.

When the animal was decapitated, the blood was collected in a glass containing potassium oxalate. The sugar content of the blood was determined by the Lewis-Benedict method (23).

The epinephrine content of the adrenal gland was determined by a color method with mercuric acetate. Comparing various color methods,

Borberg (24) proved that they can be used for the approximate determination of the epinephrine content of the adrenal gland itself. Underhill and Fine (25) and Comessatti (26) obtained a satisfactory result by the color produced by mercuric chloride. The procedure used in the present work was as follows: Both adrenals were ground in a mortar with 0.2 gram of sand and 5 cc. of physiological saline solution. The mixture was kept in a stoppered tube in a cold room for three hours. After centrifuging, 2 cc. of the upper clear part were removed to a small test tube. One cubic centimeter of saturated mercuric acetate solution was added. Protein was precipitated immediately and a pink color developed in the upper clear portion. For comparing the depth of the color, adrenalin chloride (1:1000, Parke, Davis and Company) was diluted in the proportion of 1:25, 1:30 and 1:35 with distilled water, and mixed with mercuric acetate solution in the same manner. The color obtained from the adrenal glands of normal rats was nearly as deep as that of the 1:30 dilution.

Rabbit experiments. Full-grown rabbits were fed on oats and carrots. Water was given freely. Before the experimental period, all the animals took 75 grams of carrots and 50 grams of oats per day for at least two days. For producing hyperthyroidism, fresh thyroids of pigs were used, calf's liver being used for control experiments. The two latter foods were cut into small pieces and fed to the animals by hand.

The urine was collected by compression of the bladder through the abdominal wall. The blood sample was taken from an ear vein and its sugar content was determined by the Lewis-Benedict method. The blood sugar content in the morning of the first experimental day shows the normal value, the blood sample having been taken before the first thyroid feeding. The sugar in the urine was estimated polarimetrically after removing the coloring matters and levorotatory substances with saturated mercuric acetate solution. Total nitrogen of the urine was determined by Kjeldahl's method.

THE INFLUENCE OF THYROID FEEDING UPON THE GLYCOGEN CONTENT OF THE LIVER

In Cramer and Krause's experiments (14), a glycogen content of the liver of the thyroid-fed rats was barely discernible, the amount in the control animals being on the average about 2 per cent. The amount of thyroid (sheep's) fed was described as one lobe, one-half lobe, etc.,

per day. The duration of feeding was two to eight days. Even a single administration of one lobe of thyroid seemed to be enough to diminish liver glycogen. In my experiments the amount of thyroid and other food was determined exactly. The animals seemed a little weaker after two or three days of thyroid feeding, the appetite usually being affected. Diarrhoea, which Carlson (12) and Gudernatch (27) described as a remarkable symptom after thyroid feeding, was not observed with my experimental conditions. Glycosuria was never found. In autopsy, no marked macroscopical changes were seen, excepting in one case only (Rat III in table 1) where the alimentary tract showed a slight hyperemia. In all cases the stomach and small intestine contained chyle, showing that they were in a stage of active digestion. Judging from the color reaction, the epinephrine content of the adrenal gland of the thyroid-fed rats did not differ from that of the control animals. Three rats, not recorded in the tables, took no food at the end of the thyroid period and they died in one or two days, although thyroid was omitted from the diet. The results are detailed in tables 1 and 2.

From tables 1 and 2 it will be seen that feeding fresh thyroid decreased the glycogen content of the liver markedly. This confirms the results of Cramer and Krause. The thyroid-fed animals always lost in body weight. Although in some control experiments (Rats III, IV and V in table 2) the amount of food was regulated so that it was not enough to maintain their body weight, the glycogen content of the liver remained normal. The sugar content of the blood was normal at the end of the period.

Desiccated thyroid was also fed. Neither glycosuria nor diarrhoea was present. The results are detailed in table 3.

In these cases, the regulation of the amount of food was not so strictly observed. But a decrease of glycogen content of the liver caused by thyroid feeding can be clearly seen. Autopsy showed no noteworthy macroscopical changes. Chyle was found both in the stomach and small intestine in all cases.

In a discussion of the glycogen content of the liver the extent of digestion and absorption of the food also must be taken into consideration. The decrease of the glycogen of the liver of the thyroid-fed rats can hardly be explained by the insufficient digestion and absorption of the food, because (1) no noteworthy change in the appearance of the alimentary tract could be observed; (2) the feces were

TABLE 1

The influence of fresh thyroid feeding upon the glycogen content of the liver and the sugar content of the blood

RAT	I		II		III		IV		V		VI	
Number												
Body weight, grams	154.6		196.6		220.1		154.7		198.0		184.6	
Food	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
First day	5.0	8.0	5.0	8.0	5.0	8.0	5.0	8.0	3.0	9.0	3.0	9.0
Second day	5.0	8.0	5.0	8.0	5.0	8.0	5.0	8.8	3.0	6.0	3.0	5.1
Third day	5.0	5.2	5.0	9.0	5.0	9.0	5.0	9.0	3.0	6.0	3.0	4.1
Fourth day	5.0	9.0	5.0	9.0	5.0	9.0	5.0	8.2				
Fifth day	5.0	6.6	5.0	8.2	5.0	9.0						
Loss of body weight at end of period, gram	−3.2		−8.6		−11.3		−7.2		−10.8		−13.3	
Blood sugar content, per cent	0.11		0.12		0.11		0.11		0.12		0.13	
Weight of liver, gram	6.1		8.3		7.6		5.3		5.3		6.8	
Glycogen content of liver: Milligram	7.4		13.6		22.9		trace		31.6		64.5	
Glycogen content of liver: Per cent	0.12		0.16		0.29				0.60		0.95	

TABLE 2

Control experiments. The glycogen content of the liver and the sugar content of the blood after meat feeding

RAT	I		II		III		IV		V	
Number										
Body weight, gram	153.7		213.0		182.2		194.2		145.1	
Food	Meat	Paste	Meat	Paste	Meat	Paste	Meat	Paste	Meat	Paste
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
First day	5.0	8.0	5.0	8.0	3.0	7.0	3.0	7.0	0	7.0
Second day	5.0	8.0	5.0	8.0	3.0	7.0	3.0	7.0	0	5.0
Third day	5.0	8.0	5.0	8.0	3.0	3.0	3.0	5.0	0	4.0
Fourth day	5.0	8.0	5.0	8.0						
Fifth day	5.0	8.0	5.0	8.0						
Change of body weight at end of period, gram	+15.5		+0.3		−11.1		−10.5		−3.7	
Blood sugar content, per cent	0.13				0.10		0.09		0.11	
Weight of liver, gram	6.5		8.6		6.0		6.5		6.3	
Glycogen content of liver: Milligram	322.7		344.3		165.8		247.9		218.5	
Glycogen content of liver: Per cent	4.97		4.00		2.76		3.81		3.47	

TABLE 3

The influence of desiccated thyroid feeding upon the glycogen content of the liver

RAT		I	II	III	IV	V*	VI*
	Number						
	Body Weight, gm.	222.3	247.6	310.1	187.3	237.5	211.1
Food		Thyroid + Paste	Thyroid + Paste	Thyroid + Paste	Thyroid + Paste	Egg + Paste	Egg + Paste
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
First day		7.1	11.3	12.6	11.5	14.7	9.3
Second day		9.8	17.6	7.8	11.6	11.8	13.5
Third day		5.3	13.5	6.6	4.7	9.0	12.7
Fourth day		6.6	6.4	9.9	6.4	9.0	9.0
Change of body weight at end of period, gram		−17.5	−24.0	−22.6	−19.8	+0.8	+14.4
Weight of liver, gram		7.8	8.3	7.7	8.9	7.4	7.5
Glycogen content of liver	Milligram	40.7	trace	trace	trace	148.7	290.2
	Per cent	0.52				2.01	3.07

* Control experiments.

normal both in amount and character; (3) there was also poor glycogen formation in the liver when dextrose was parenterally administered. The latter point will be discussed in a later section of this paper.

THE GLYCOGEN CONTENT OF THE LIVER OF THYROID-FED RATS AFTER OMITTING THYROID FROM THE DIET

Kojima (28) found that after thyroid feeding pronounced histological changes are produced in the pancreas of rats. The question, whether the decrease of glycogen content of the liver bears some relation to the changes in the pancreas or is due to hypersecretion of the adrenal glands or to other factors, is yet to be decided. But it is interesting to know whether the changes, wherever they may be, resulting from thyroid feeding and causing the decrease of glycogen content of the liver, can or cannot be repaired very easily by omitting thyroid from the diet.

Four rats were fed first on fresh thyroid and the paste for a few days, and then on thyroid-free diet. Two, three or thirteen days after omitting thyroid from the diet, the glycogen content of the liver was determined. The results are detailed in table 4.

TABLE 4

The glycogen content of the liver of thyroid-fed rats after omitting thyroid from the diet

RAT		I			II			III			IV		
Number													
Body weight, grams		251.5			141.3			149.1			155.4		
Food			Thyroid	Paste		Thyroid	Paste		Thyroid	Paste		Thyroid	Paste
First period with thyroid		*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*
		1	3.0	9.0	1	3.0	9.0	1	3.0	6.0	1	5.0	9.0
		2	3.0	8.2	2	3.0	5.2	2	3.0	6.1	2	5.0	6.6
		3	3.0	9.0	3	3.0	4.5	3	3.0	6.5	3	5.0	3.7
											4	5.0	5.0
											For 13 days biscuit was given freely		
			Meat	Paste		Meat	Paste		Meat	Paste		Meat	Paste
Second period without thyroid		*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*
		1	3.0	8.0	1	3.0	5.0	1	0	6.0	14	3.0	7.0
		2	3.0	8.0	2	3.0	5.0	2	3.0	4.8	15	0.7	6.0
								3	3.0	5.4			
Change of body weight at end of second period, gram		−17.7			−8.0			−12.5			+1.3		
Weight of liver, gram		7.7			5.8			5.0			6.5		
Glycogen content of liver	Milligram	180.9			209.1			187.4			302.3		
	Per cent	2.35			3.61			3.75			4.65		

From table 4, it will be seen that glycogen is very easily stored in the liver after omitting thyroid from the diet, notwithstanding that the loss of body weight still remains. It is scarcely probable, therefore, that the changes causing the loss of liver glycogen are of a serious morphological nature.

THE FORMATION OF LIVER GLYCOGEN BY PARENTERAL ADMINISTRATION OF DEXTROSE IN THYROID-FED RATS

Grube (29) and many other investigators found that perfusion of the liver with blood or physiological saline solution containing excessive dextrose (1 per cent or more) can increase the glycogen content of the

liver in a few hours. Gumprecht (30) demonstrated that subcutaneous injection of dextrose into fasted rabbits can cause glycogen storage in the liver as high as 3.9 per cent. He injected 20 grams of dextrose in eight to nine hours. Before going on with the experiments on thyroid-fed rats, Gumprecht's experiments were repeated with fasted rats. After a certain period of fasting a 10 per cent dextrose solution, sterilized by boiling, was injected subcutaneously and intraperitoneally into the animals. The subcutaneous injection (7.5 to 15.0 cc.) was performed three hours before and the intraperitoneal injection (5.0 to 7.5 cc.) two hours before decapitation. The glycogen content of the liver was increased distinctly by this procedure. On the average, the liver of the fasted rats into which dextrose was injected contained 1.28 per cent glycogen, the liver of the control animals showing only 0.31 per cent glycogen.

Fresh pig thyroid was given to four rats for three days and then dextrose was injected as described for fasted rats. The average of glycogen content of the liver was 0.7 per cent. The same experimental conditions of pure thyroid feeding was performed in Rats V and VI in table 1. The average of glycogen content of the liver of these last mentioned rats was 0.77 per cent. Storage of liver glycogen seems, therefore, to be affected by thyroid feeding. The details can be seen in tables 5 and 6.

TABLE 5

The formation of liver glycogen by parenteral administration of dextrose in fasted rats

RAT	No.	I	II	III	IV	V	VI
	Body weight, grams	152.2	166.8	196.4	100.8	149.3	175.7
Duration of fasting, hours		48	57	72	48	57	72
Loss of body weight, gram		−19.2	−17.7	−19.0	−9.5	−14.4	−27.9
Dextrose injection		none	none	none	0.75 gm. subcutan. 0.5 gm. intraperit.	1.5 gm. subcutan. 0.75 gm. intraperit.	1.5 gm. subcutan. 0.75 gm. intraperit.
Weight of liver, gram		3.8	4.2	5.1	3.1	4.6	6.0
Glycogen content of liver	Milligram	13.6	trace	29.3	35.8	63.2	78.7
	Per cent	0.36		0.58	1.16	1.38	1.31

TABLE 6

The influence of parenteral administration of dextrose upon the glycogen content of the liver of thyroid-fed rats

Dextrose injected { 1.5 gm. subcutaneously 3 hours / 0.75 gm. intraperitoneally 2 hours } before decapitation

RAT		I		II		III		IV	
	No.	I		II		III		IV	
	Body weight	195.7		187.0		262.2		166.1	
Food		Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
First day		3.0	4.7	3.0	9.0	3.0	9.0	3.0	8.1
Second day		3.0	4.7	3.0	2.6	3.0	9.0	3.0	4.0
Third day		3.0	4.5	3.0	3.8	3.0	9.0	3.0	5.5
Loss of body weight at end of period, gram		−12.0		−11.8		−14.2		−9.0	
Weight of liver, gram		5.8		5.1		6.3		5.0	
Glycogen content of liver	Milligram	54.3		5.6		60.0		40.5	
	Per cent	0.95		0.11		0.95		0.81	

As thyroid feeding increases oxidative process in the organism, it may be that the sugar injected was utilized immediately and had no chance to form glycogen. Another explanation may be that the thyroid-fed animal eliminates the excessive sugar much quicker than the normal animal, and thus cannot get any chance to store the sugar as glycogen. But the rabbit experiments for the carbohydrate tolerance after thyroid feeding, which will be described shortly, seem to make these explanations improbable. Cramer and Krause (14) described that after thyroid feeding, besides the loss of glycogen in the liver, glycogen content of other parts of the body sometimes decreased. The loss of liver glycogen and the inability of the liver to store dextrose as glycogen may be caused either by a disturbance of the glycogenetic function of the liver or by an increased glycogenolytic function of the liver. Starkenstein (31) and Wohlgemuth (32) could not demonstrate any change of diastatic action of the liver and blood after epinephrine glycosuria and piqûre. Macleod and Pearce (33) reported that stimulation of the splanchnic nerve, which caused a marked increase in the reducing power of the blood of the vena cava opposite the liver, did not cause any increase in the glycogenolytic power of liver extracts. Though pancreas diabetes shows a low glycogen content of the liver.

Nishi (34) proved by his perfusion experiments that the glycogenetic function of the liver is not affected in this disease. The complicated relation of the liver, thyroid, pancreas, adrenal, sympathetic nervous system and sugar center has been examined by numerous investigators (35) (36). But many points are not yet decided. Eppinger and his coworkers showed that thyroid preparations stimulate the sympathetic nervous system. The hypothesis, suggested by Cramer (19), that the influence of thyroid feeding upon liver glycogen is caused by the mediation of the adrenal glands, may be very probable. But for solving such a problem further investigations are necessary. An intimate relation between the adrenal glands and the glycogenolysis in the liver was demonstrated by Macleod and Pearce (37). On the other hand, though Fränkel (38) and Bröking and Trendelenburg (39) reported that they found an increased epinephrine content of the blood in Basedow's disease, Gottlieb (40) tried to explain the results of those investigators without assuming any excessive epinephrine in the blood. Neubauer and Porges (41) found that phosphorus, which causes the loss of liver glycogen, makes also the adrenal glands lose their chromaffin property, and that frequent adrenal injections are sometimes able to prevent the loss of liver glycogen resulting from phosphorus poisoning. On the other hand, Underhill and Fine (25) found no remarkable change of epinephrine content of the adrenal glands after hydrazine poisoning, which usually causes the loss of liver glycogen as well as phosphorus poisoning.

THE INFLUENCE OF THYROID FEEDING UPON THE SUGAR CONTENT OF THE BLOOD AND THE UTILIZATION OF DEXTROSE PARENTERALLY ADMINISTERED

Though it seems to be believed by many investigators that the tolerance for carbohydrate is increased in hypothyroidism and is lowered in hyperthyroidism (42) there are found some contradictory reports on this problem. The complicated and probably antagonistic relation between the thyroid and parathyroid glands, and varying susceptibility of different kinds of animals must be kept in mind. Observations in Basedow's disease cannot be compared unconditionally with those of experimental hyperthyroidism. Cramer and Krause (14) could not find any glycosuria in rats after thyroid feeding. In their dog experiment only a slight lowering of the carbohydrate tolerance

TABLE 7

The influence of thyroid feeding upon the sugar content of the blood and the utilization of dextrose parenterally administered

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT		URINE		DEXTROSE	
			Thyroid	Carrots	Oats	9 a. m.	4 p. m.	Volume	Total nitrogen	Injected intraperitoneally	Recovered in urine
		kgm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*cc.*	*gm.*	*gm.*	*gm.*
I*	1	2.14	10	75	50	0.10					
	2		15	75	31		0.11	110	0.823		
	3		15	75	5	0.11	0.10	105	0.914		
	4		15	33	5	0.11	0.11	74	0.979		
	5	1.75	15	16	0	0.08				12.33	
II	1	2.74	15	75	50	0.10	0.10	62	0.893		
	2		15	75	50	0.11	0.09	125	1.088		
	3		15	75	7	0.11	0.11	50	0.870		
	4	2.46	15	40	0	0.12		111	1.426	12.33	2.19
III	1	2.40	15	75	50	0.11	0.10	107	0.891		
	2		15	75	41	0.12	0.12	127	0.836		
	3	2.27	15	75	0	0.11		144	1.015	12.33	2.60

* The animal was found dead in the morning of the 6th day. The urine obtained measured 32 cc. and contained 0.144 gm. nitrogen and a trace of reducing sugar.

TABLE 8

Control experiments. The sugar content of the blood and the utilization of dextrose parenterally administered in calf's liver fed rabbits.

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT		URINE		DEXTROSE	
			LIVER	CARROTS	OATS	9 a. m.	4 p. m.	Volume	Total nitrogen	Injected intraperitoneally	Recovered in urine
		kgm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*cc.*	*gm.*	*gm.*	*gm.*
I	1	2.34	15	75	50	0.09	0.10	141	0.745		
	2		15	75	50	0.10	0.09	167	0.880		
	3		15	75	50	0.11	0.10	71	0.697		
	4	2.32	15	75	31	0.11		65	0.614	12.33	2.25
II	1	2.68	15	75	50	0.09	0.09	143	1.294		
	2		15	75	50	0.11	0.09	107	0.882		
	3		15	75	50	0.08	0.10	111	0.761		
	4	2.54	15	75	0	0.10		100	0.970	12.33	2.45

was noticed. Böe (43) could not demonstrate any remarkable hyperglycemia in rabbits after injection or feeding of thyroid preparations. In my experiments with fresh thyroid-fed rabbits, the sugar content of the blood and the utilization limit of dextrose intraperitoneally administered were examined. A 10 per cent dextrose solution, sterilized by heat, was injected in the peritoneal cavity without anesthesia. In no case was glycosuria observed until dextrose injection. In tables 7 and 8 the details are shown.

From these tables it will be seen that the sugar content of the blood and the utilization limit of dextrose parenterally administered are not affected by thyroid feeding.

THE INFLUENCE OF THYROID FEEDING UPON THE EPINEPHRINE HYPERGLYCEMIA AND GLYCOSURIA

As regards the influence of thyroidectomy upon epinephrine glycosuria, contrary results have been reported. (Eppinger, Falta and Rudinger (6) ,(7), Underhill and Hilditch (8), Underhill (44), Pick and Pineles (9), Grey and de Sautelle (45)). Böe could not demonstrate any influence of hyper- or hypothyroidism upon epinephrine hyperglycemia. My experiments in this direction are detailed in tables 9 and 10.

Epinephrine (adrenalin chloride 1:1000, Parke, Davis and Company) was injected subcutaneously in the proportion of 1 milligram per kilo of body weight. In no cases was glycosuria demonstrated on the first and second experimental day. As the thyroid-fed animals became very weak, the urine of the last experimental day was collected six and one-half hours after epinephrine injection. The animals were alive two hours later, but found dead the next morning.

From tables 9 and 10, it will be seen that feeding of fresh thyroid does not affect epinephrine hyperglycemia or glycosuria markedly. These results are in harmony with those of Böe's experiments.

SUMMARY

Fresh thyroid gland of pigs or desiccated thyroid (Parke, Davis and Company) administered by mouth in doses of 3 to 5 grams (fresh) or 0.5 to 1.7 gram (desiccated) per day, decreased the glycogen content of the liver of white rats distinctly in three to five days. Control animals, fed on the same diet with the addition of muscle tissue or

TABLE 9

The influence of thyroid feeding upon the epinephrine hyperglycemia and glycosuria

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT (PERCENTAGE)				URINE		
						Before epinephrine injection	Hours after epinephrine injection					
			Thyroid	Carrots	Oats		2	4	6	Volume	Total nitrogen	Sugar
		kgm.	*gm.*	*gm.*	*gm.*					*cc.*	*gm.*	*gm.*
I	1	2.60	15	75	50					67	1.076	0
	2		15	75	33					118	1.500	0
	3	2.38	15	75	0	0.11	0.46	0.40	0.26	104*	0.605	4.44
II	1	2.44	15	75	50					116	0.953	0
	2		15	75	50					164	0.926	0
	3	2.26	15	75	0	0.10	0.40	0.32	0.24	92*	0.648	3.19

* The urine was collected from 9 a.m. to 6.30 p.m. Epinephrine was injected at 12 m.

TABLE 10

Control experiments. The epinephrine hyperglycemia and glycosuria in calf's liver fed rabbits

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT (PERCENTAGE)				URINE		
						Before epinephrine injection	Hours after epinephrine injection					
			Liver	Carrots	Oats		2	4	6	Volume	Total nitrogen	Sugar
		kgm.	*gm.*	*gm.*	*gm.*					*cc.*	*gm.*	*gm.*
I	1	2.54	15	75	50					110	0.976	0
	2		15	75	50					97	0.870	0
	3	2.48	15	75	0	0.12	0.31	0.33	0.18	153*	0.712	4.72
II	1	2.80	15	75	50					33	0.942	0
	2		15	75	50					60	1.150	0
	3	2.88	15	75	0	0.10	0.37	0.30	0.17	144*	0.879	4.53

* The urine was collected from 9 a.m. to 6.30 p.m. Epinephrine was injected at 12 m.

egg, do not show any such change, even when the food amount is regulated so that they lose as much in body weight as the thyroid-fed animals.

The influence of thyroid feeding upon liver glycogen can be very easily removed by omitting thyroid from the diet. The liver shows its normal glycogen content two or three days after the cessation of thyroid administration, even when the loss of body weight has not been regained. This phenomenon seems to show that the changes resulting from thyroid feeding and causing the loss of liver glycogen, are not of a serious morphological nature.

When dextrose is introduced parenterally to fasted rats which show a very low glycogen content of the liver the amount of liver glycogen increases markedly in a few hours. This does not seem to be the case in the thyroid-fed rats.

Experimental hyperthyroidism does not change the sugar content of the blood in either rats or rabbits.

Spontaneous glycosuria does not result from thyroid feeding in either rats or rabbits.

The tolerance of thyroid-fed rabbits for dextrose, parenterally administered, does not differ from that of normal animals.

Nearly the same degree of hyperglycemia and glycosuria can be induced by epinephrine injection in thyroid-fed as in control rabbits.

The adrenal gland of thyroid-fed rats contains approximately the same amount of epinephrine as that of normal rats.

I desire to express my thanks to Prof. Frank P. Underhill, to whom I am greatly indebted for his suggestions, help and criticism; also to Prof. Lafayette B. Mendel for his kind advice.

BIBLIOGRAPHY

(1) Flesch: Beitr. z. klin. Chirur., 1912, lxxxii, 236.
(2) Schulze: Beitr. z. klin. Chirur., 1912, lxxxii, 207.
(3) Hirsch: Zeitschr. f. exper. Path. u. Therap., 1906, iii, 393.
(4) Underhill and Saiki: Journ. Biol. Chem., 1908, l, 225.
(5) Hirsch: Zeitschr. f. exper. Path. u. Therap., 1908, v, 233.
(6) Eppinger, Falta and Rudinger: Zeitschr. f. klin. Med., 1908, lxvi, 1.
(7) Eppinger, Falta and Rudinger: Zeitschr. f. klin. Med., 1909, lxvii, 380.
(8) Underhill and Hilditch: This Journal, 1909, xxv, 66.
(9) Pick and Pineles: Biochem. Zeitschr., 1908, xii, 473.
(10) McCurdy: Journ. Exper. Med., 1909, xi, 798.
(11) Miura: Biochem. Zeitschr., 1913, li, 423.
(12) Carlson, Rooks and McKie: This Journal, 1912, xxx, 127.
(13) French: This Journal, 1912, xxx, 56.
(14) Cramer and Krause: Proc. Roy. Soc. B., 1913, lxxxvi, 550.

(15) CRAMER AND MCCALL: Journ. Physiol., 1916, l, xxxvi.
(16) SCHWARZ: Pflüger's Arch. f. Physiol., 1910, cxxxiv, 259.
(17) POLLACK: Arch. f. exper. Path. u. Pharm., 1909, lxi, 149.
(18) KAHN AND STARKENSTEIN: Pflüger's Arch. f. Physiol., 1911, cxxxix, 181.
(19) CRAMER: Journ. Physiol., 1916, l, xxxviii.
(20) OSBORNE AND MENDEL: Feeding experiments with isolated food substances, Washington, 1911, 12.
(21) PFLÜGER: Pflüger's Arch. f. Physiol., 1903, xcvi, 1.
(22) PFLÜGER: Pflüger's Arch. f. Physiol., 1909, cxxix, 362.
(23) LEWIS AND BENEDICT: Journ. Biol. Chem., 1915, xx, 61.
(24) BORBERG: Skand. Arch. f. Physiol., 1912, xxvii, 347.
(25) UNDERHILL AND FINE: Journ. Biol. Chem., 1911, x, 271.
(26) COMESSATTI: Arch. f. exper. Path. u. Pharm., 1910, lxii, 190.
(27) GUDERNATCH: This Journal, 1914, xxxvi, 370.
(28) KOJIMA: Journ. Physiol., 1916, l, xlvi.
(29) GRUBE: Journ. Physiol., 1903, xxix, 276.
(30) GUMPRECHT: Verhandl. d. Kong. f. inn. Med., 1898, xvi, 124.
(31) STARKENSTEIN: Biochem. Zeitschr., 1910, xxiv, 191.
(32) WOHLGEMUTH: Biochem. Zeitschr., 1909, xxi, 381.
(33) MACLEOD AND PEARCE: This Journal, 1911, xxviii, 403.
(34) NISHI: Arch. f. exper. Path. u. Pharm., 1910, lxii, 170.
(35) KAHN: Pflüger's Arch. f. Physiol., 1911, cxl, 209.
(36) KING: Journ. Exper. Med., 1909, xi, 665.
(37) MACLEOD AND PEARCE: This Journal, 1911, xxix, 419.
(38) FRÄNKEL: Arch. f. exper. Path. u. Pharm., 1909, lx, 395.
(39) BRÖKING AND TRENDELENBERG: Deutsch. Arch. f. klin. Med., 1911, ciii, 168.
(40) GOTTLIEB: Vers. d. Naturf. u. Ärzte, Karlsruhe, 1911 (ref. by v. Fürth Probleme der physiol. u. path. chem., Leipzig, 1912, i, 457).
(41) NEUBAUER AND PORGES: Biochem. Zeitschr. 1911, xxxii, 290.
(42) GEYELIN: Arch. Int. Med., 1915, xvi, 975.
(43) BÖE: Biochem. Zeitschr., 1914, lxiv, 450.
(44) UNDERHILL: This Journal, 1910, xxvii, 331.
(45) GREY AND DE SAUTELLE: Journ. Exper. Med., 1909, xi, 659.

VARIATIONS IN IRRITABILITY OF THE REFLEX ARC

III. Flexion Reflex Variations, Compared with Those of the Nerve-Muscle Preparation

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The object of this research was to compare the variations in threshold for faradic stimulation of the flexion reflex in the spinal cat, with similar variations in the nerve-muscle preparation, using the same muscle in each case, and keeping other conditions as uniform as possible, while the two sets of determinations were being made. The results were intended primarily to serve as a control on further quantitative studies on reflexes, in which it was important to know to what minimal values the variations in reflex thresholds would be reduced under the most favorable conditions of stimulation; that is, in animals unaffected by drugs or other experimental procedures except those incident to spinal transection. In other words, an answer was sought to the question: How smooth will the curve plotted for flexion reflex thresholds appear compared with the curve for nerve-muscle thresholds, when these curves are obtained from the same muscle, and from an animal whose tissues are in as nearly normal condition as possible?

The spinal cat was prepared by a modification of Sherrington's method, described in detail in a previous paper,[1] in brief, the carotids were tied, brain pithed, artificial respiration supplied and the animal kept at approximately normal body temperature by an electric heating pad. To avoid interference from the scratch reflex, the cord was cut in the neighborhood of the tenth dorsal vertebra. The femur and foot were held in clamps, immobilizing the leg at knee and ankle; the tendon of the tibialis anticus muscle was cut at its distal end, and a thread carried from it in the direction of its normal pull to a myograph lever whose effective tension on the muscle was about 35 grams. The peroneus longus muscle was excised to a point above the entrance of

[1] Porter: This Journal, 1912, xxxi, 141.

its motor nerve, and the femoral and the hamstring nerves were sectioned, thus paralyzing most of the muscles concerned in the flexion reflex aside from the tibialis anticus. An electrode of a form later to be described, was placed on the posterior tibial nerve and the break induction shock necessary to cause the least detectible movement of the writing point of the myograph lever on the moving smoked drum was determined; the lever magnified the movement of the muscle twice. Readings were made approximately once a minute for from one-half to one hour; the peroneus nerve in the thigh was then sectioned and the electrode applied to its distal end, giving a nerve-muscle preparation with the same muscle as before, and a second series of threshold determinations made.

Several attempts were made to secure simultaneous observations on reflex and nerve-muscle, stimulating the peroneal nerve in the hip without cutting it, using a modification of the electrode mentioned above, but conductivity was always diminished in the neighborhood of the electrode, so the attempts were abandoned. The plan of obtaining simultaneous records from homologous muscles on the two legs was not tried. The inductorium was a large one with secondary 13 cm. long and 6 cm. in diameter; it was calibrated by comparison with a coil already calibrated by the Martin method;[2] a part of the calibration curve is shown in figure 1. Secondary coil positions are measured from complete superposition over the primary, which is position 0. It is evident that secondary coil positions from about 25 outward, will give the greatest accuracy in quantitative work, since a relatively large movement of the secondary yields only a small increment in the value $\frac{M}{L}$; hence the secondary has been used within these limits in the present research. The Z units referred to in this paper are obtained

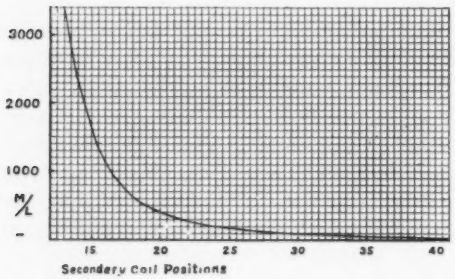


Fig. 1. Calibration curve for the inductorium used. (Martin calibration.)

[2] Martin: The measurement of induction shocks, New York, 1912.

by multiplying the value $\frac{M}{L}$ for any given secondary position, by the current in amperes through the primary coil; the latter value has been 0.1 ampere in most experiments.

The threshold of a reflex determined by the method I have described may be affected by a number of possible variables: (1) The nerve has abnormal conditions of irritability near the point of ligation or section. Gotch[3] found alterations in the electrical response 8 mm. from the point of section; Forbes[4] reports similar results. (2) The electrodes at their point of application may introduce variables. I had much difficulty in a previous research[5] in using the quantitative method, because of the short-circuiting of a varying fraction of the stimulating shock by the minute drop of liquid which collected between the platinum wires of the Sherrington shielded electrode;[6] the removal of this drop gave an apparent lowering of threshold of from 0.7 to 1.5 Z units. In justice to the Sherrington form it should be said that, if it be carefully applied so that the nerve is not compressed, and unless the minutest variations in threshold are to be followed, it can be used entirely successfully; for example, I have determined the threshold with it at the beginning of an experiment and found it 2.4 Z units; six and a half hours later the threshold was 3.7 Z units, so that no serious local impairment could have been caused by its application. (3) The central nervous connections are held by Sherrington[7] to be responsible for the greater variability in threshold of the reflex arc as compared with the nerve trunk. (4) The nerve trunk and (5) the myo-neural junction may vary in conductivity.[8] (6) The muscle fibers themselves may conceivably vary in excitability. It was my purpose to eliminate variations at the point of stimulation as far as possible, and then to compare the variations in threshold of the group of structures comprising the reflex arc with similar variations in the nerve-muscle group, intending thereby to demonstrate the variability introduced by the central connections between neurone and neurone. In such a study it would be desirable to utilize an arc with the fewest possible central connections, and

[3] Gotch: Journ. Physiol., 1902, xxviii, 32.

[4] Forbes and Gregg: This Journal, 1915, xxxix, 172.

[5] Porter: This Journal, 1915, xxxvi, 171.

[6] Sherrington: Journ. Physiol., 1909, xxxviii, 375.

[7] Sherrington: The integrative action of the nervous system, New Haven, 1906, 14.

[8] Cf. Burridge: Journ. Physiol., 1911, xli, 285.

those of as low a threshold as possible, that the results might represent minimal variations in reflex arc conduction; there are observations which indicate that the flexion reflex, and in particular the contraction of the tibialis anticus muscle, approximates these requirements. First, the flexion reflex is one of the few to respond to weak single shocks;—crossed extension can be readily elicited by single shocks but in my experience commonly has a higher threshold than flexion.[9] Second, among the muscles concerned in the flexion reflex Sherrington[10] found some with higher thresholds than others, but none with a lower than tibialis anticus; I have found the same result. In an experiment to test the point the tendons of insertion were cut, the muscles isolated as much as possible from each other and contraction observed directly while the tendon was held in the hand; the thresholds were as follows in Z units: tibialis anticus 4.7; peroneus longus 5.1; extensor longus digitorum 11.8; biceps (posterior portion) 4.9; tensor fasciae latae 8.1; semitendinosus 4.7; sartorius (portion inserted on patella) 5.5; gracilis 5.2. These figures are averages of from two to seven determinations on each muscle; the stimulating electrode was on the posterior tibial nerve and single break shocks were used. Finally, if speed of transmission be a criterion of the number of central connections concerned, then the flexion reflex has few, for Sherrington[11] found with strong shocks little difference between the rate through the reflex arc and through a corresponding length of nerve trunk.

For the purpose of avoiding the difficulties mentioned above incident to the use of the Sherrington electrodes a form of liquid electrode suggested by those used by Lucas[12] has been employed; it is shown in longitudinal section, once and a half natural size, in figure 2. A glass tube *b* with a constricted neck *i* has a short piece of rubber tube *c* drawn over its end tightly for a short distance; closing the lumen of the rubber tube is a glass ball *e* which has been melted on to a platinum wire *f* ending in a loop; a copper wire *g* is soldered to the platinum wire. The nerve *a* has a ligature *dd'* attached to its end; the space *h* is filled with a paste of kaolin in Ringer's solution, mixed to the consistency of cream. Water distilled from glass and chemically pure salts are used

[9] Porter: Loc. cit.

[10] Sherrington: Journ. Physiol., 1910, xl, 28.

[11] Sherrington: Integrative action of the nervous system, New Haven, 1906, 19.

[12] Lucas: Journ. Physiol., 1913, xlvi, Proc. p. xxxii; 1913, xlvi, 480; 1908, xxxvii, 114; 1906, xxxiv, 375.

in making this up; the kaolin is Baker's, washed twice in distilled water and twice in Ringer's solution. Ringer's solution alone was not used in the tube *b*, because of the ease with which it permitted bubbles to enter. The same paste surrounds the nerve at its emergence from the tube at *i*. The wire *g* is connected to one terminal of the induction coil; the other terminal is connected to a platinum needle thrust deeply into the shoulder muscles (later experiments) or to the tracheal cannula (earlier experiments); *f* is made the cathode. The current in passing from one electrode to the other reaches its greatest density at *i* and stimulates the nerve at that point, as it does at the corresponding points in the Lucas models.

The electrode is applied as follows: the nerve—posterior tibial, for example,—is carefully separated from surrounding tissues for a distance of 4 or 5 cm., is ligated, and cut at the distal end; a glass tube is selected from among several of different sizes kept on hand, with an opening at *i* as nearly as possible of the diameter of the nerve; the ligature is

Fig. 2. Liquid electrode, once and a half natural size; description in text.

passed through the tube and the nerve drawn in after it to a distance of about 1.5 cm. With a fine-pointed pipette the glass tube is now filled with the kaolin-Ringer mixture; the lumen of the rubber tube surrounding the platinum loop has previously been filled with the mixture, and the rubber tube is now forced over the glass one, thus holding the ligature tightly, preventing the nerve from slipping out. If bubbles are by accident included in the glass tube, the glass ball *e* is pinched forward with the fingers until they are forced out at *i*. A thread is passed through a small portion of the tibialis posticus, or neighboring muscles, and tied about the tube at *i*, care being taken that the nerve is neither pulled, nor forced to make an angle at *i*, which would compress it at that point, since a relatively slight pressure there raises the threshold and necessitates rearrangement of the electrode. The nerve is surrounded with the kaolin-Ringer paste at the point of its emergence from the tube; the rubber tube is made long

enough to pass out of the wound, the edges of which are closed over the electrode with spring clips. In applying the electrode to the peroneal nerve in the thigh, the same procedure is followed, except that in some cases it has been found unnecessary to attach the electrode to the underlying muscles. The peroneal nerve is a part of the sciatic trunk at this point and must be dissected away from it; when thus separated its size is nearly the same as that of the posterior tibial, and usually the same electrode has been used for both. A nerve held in an electrode of the type described has had its blood supply seriously interfered with, but otherwise it is in practically a normal environment; it is bathed in Ringer's solution, and at the point of stimulation the liquid,

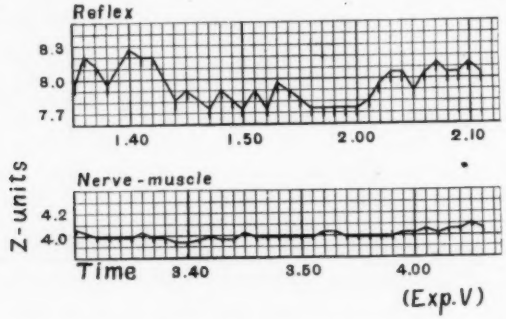


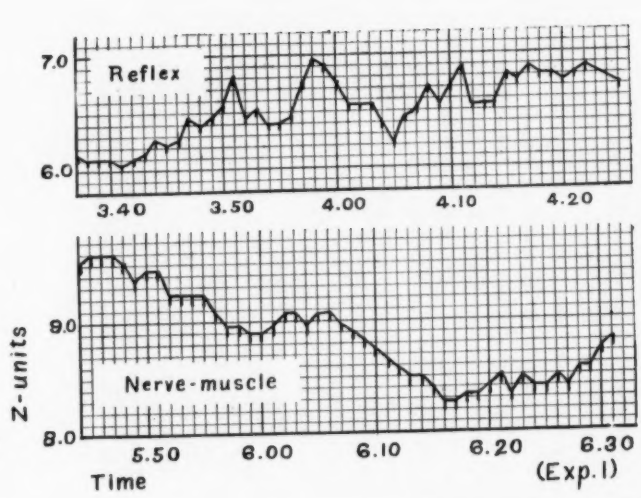
Fig. 3. Variations in threshold of reflex and nerve-muscle preparations in the spinal cat, using the same muscle, tibialis anticus, in each case. Alterations in threshold between successive readings greater for reflex than for nerve-muscle. Changes in direction more frequent in the reflex curve than in the nerve-muscle curve. Abscissae; time; ordinates, Z units (Experiment V).

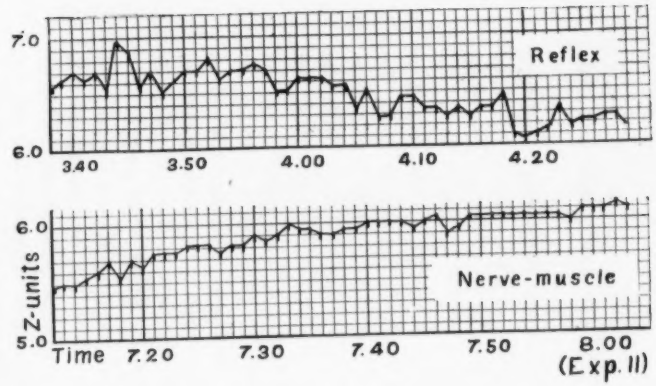
and therefore the resistance, remains constant except for possible changes in the resistance of the nerve itself; the ligated end is presumably so far away as not to be able to influence irritability at the point of stimulation.

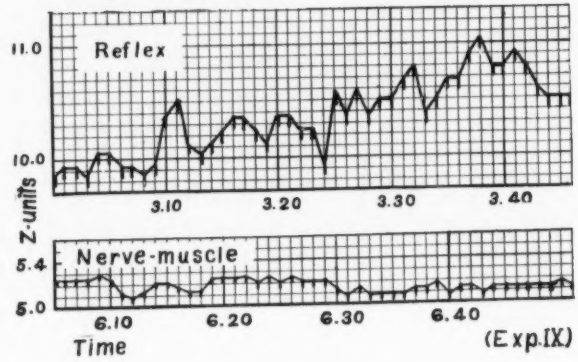
In determining the threshold the secondary coil was approximated to the primary by 1 mm. increments, while the myograph lever wrote on a moving drum, and the point at which the least detectible movement of the lever occurred was taken as the threshold, when this was confirmed by a second shock three to five seconds later. I have not found that an interval as short as this between shocks affected the threshold. Determina-

tions were made once a minute, in most cases, and continued for periods of twenty minutes to an hour, the series of nerve-muscle readings following those for the reflex as promptly as possible; data from an experiment are shown plotted in figure 3. The accuracy of the determinations in this and other figures is indicated by the length of the short vertical lines; in figure 3, for example, the threshold for the nerve-muscle was determined with an accuracy of 0.04 Z unit, corresponding to a movement of the secondary coil of 1 mm. with the secondary out 39 cm. from complete superposition over the primary, and 0.1 Amp. through the primary. From figure 3 it is evident that the alterations in threshold between successive readings is greater for reflex than for nerve-muscle, and the direction of change towards a higher or lower threshold alters oftener for reflex than for nerve-muscle, making the curve for the latter the more irregular of the two. Curves on a smaller scale are given in figure 4 for other experiments. No reason could be assigned for the gradual but pronounced lowering of nerve-muscle thresholds in experiments I and III, amounting to 2 Z units in experiment III, or more than ten times the maximum change in any minute; in contrast, the nerve-muscle of experiment V (fig. 3) altered a total of only 0.16 Z units during thirty-six minutes (highest 4.10, lowest 3.94 Z units) or about three times the maximum change in any minute (0.05 Z unit). The relatively high nerve-muscle threshold in both these experiments may have been due to poorly fitting electrodes, which allowed part of the current to be short circuited. In experiment IX the movement elicited was flexion at knee, instead of contraction of the isolated tibialis anticus muscle. Table 1 gives a summary of two important features for all the successful experiments; the difference in threshold between successive readings one minute apart (columns 2 and 3) is from 1.6 to 7.6 times as great for reflex as for nerve-muscle preparations; and the number of "peaks" in the reflex curve is greater in each experiment than the number in the nerve-muscle curve (columns 4 and 5), which is the quantitative indication that the nerve-muscle curve is the smoother of the two.

At times, for stretches of several minutes, the nerve-muscle has shown no alteration in threshold within the limits of error of the determination (fig. 3 and fig. 4, experiments II, III and IX). The reflex has never remained as steady as this; the longest period of unaltered threshold I have found for reflex has been four minutes and for nerve-muscle eight minutes. The frog nerve-muscle preparation removed


Reflex
7.0
6.0
3.40
3.50
4.00
4.10
4.20
Nerve-muscle
Z-units
9.0
8.0
5.50
6.00
6.10
6.20
6.30
Time
(Exp. I)


Reflex
7.0
6.0
3.40
3.50
4.00
4.10
4.20
Nerve-muscle
Z-units
6.0
5.0
Time
7.20
7.30
7.40
7.50
8.00
(Exp. II)


Reflex
11.0
10.0
3.10
3.20
3.30
3.40
Z-units
Nerve-muscle
5.4
5.0
6.10
6.20
6.30
6.40
Time
(Exp. IX)

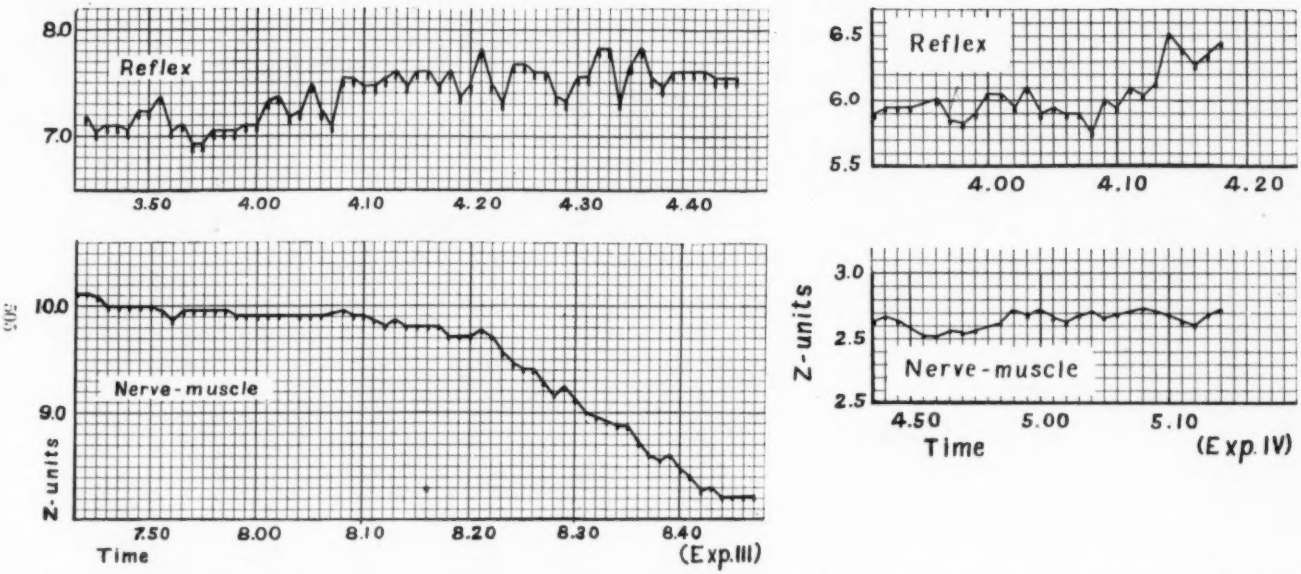


Fig. 4. Variations in threshold of reflex and nerve-muscle preparations; five experiments. Flexion at knee in Experiment IX; in all others, contraction of tibialis anticus muscle.

from the body was reported by v. Fleischl[13] many years ago as having a steady threshold, with so little variation, in fact, that it has been used by him and others[14] in calibrating the induction coil. It could hardly

TABLE 1

EXPERIMENT NUMBER	MAXIMUM VARIATIONS IN THRESHOLD IN Z UNITS, BETWEEN SUCCESSIVE DETERMINATIONS ONE MINUTE APART.		NUMBER OF "PEAKS" IN THE CURVES FOR REFLEX AND NERVE-MUSCLE RESPECTIVELY, FOR EQUAL LENGTHS OF TIME. (THE MEASURE OF SMOOTHNESS OF THE CURVES.)	
	Reflex.	Nerve-muscle.	Reflex.	Nerve-muscle.
I	3.5	2.0	10	7
II	4.3	1.5	13	9
III	4.5	1.5	14	7
IV	4.0	1.0	7	6
V	3.8	0.5	9	6
VI	2.5	0.9*	11	6
VII	2.8			
VIII	3.0			

* Exclusive of the change shown at 4.31, figure 4.

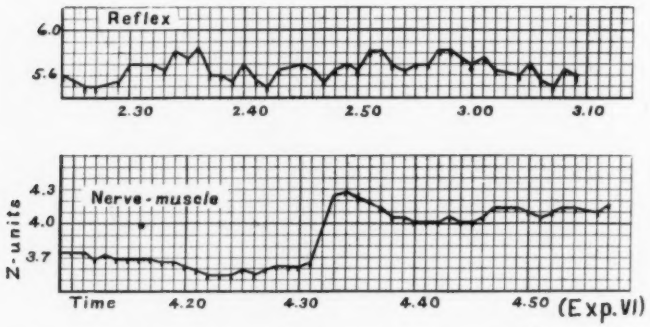


Fig. 5. Threshold of reflex and nerve-muscle preparations compared. Rise in threshold of the latter without apparent cause at 4.32 (Experiment VI).

be expected that the nerve-muscle preparation in the body of the cat would retain so constant a threshold. The frog preparation removed from the body is presumably undergoing a steady deterioration; the

[13] v. Fleischl: Sitzungsberichte der Königlichen Akademie der Wissenschaften, Wien, 1875, Bd. lxxii, Abt. III, 41.

[14] Martin: Loc. cit.

cat tissue, with intact circulation, might well show improvement as well as deterioration, dependent on alterations in blood supply or blood content. Gruber,[15] using height of contraction as an index to the condition of the nerve-muscle preparation, finds prompt improvement on increasing blood pressure. I have seen sudden changes in threshold at times without obvious reason therefor, figure 5 shows a case; just before the sudden rise in threshold at 4.31 the forelegs of the animal were strongly extended, suggesting partial asphyxia, but artificial respiration was proceeding as usual and no other experimental procedure was altered; stability of threshold in the nerve-muscle preparaton, therefore, while the rule, cannot be counted on with certainty.

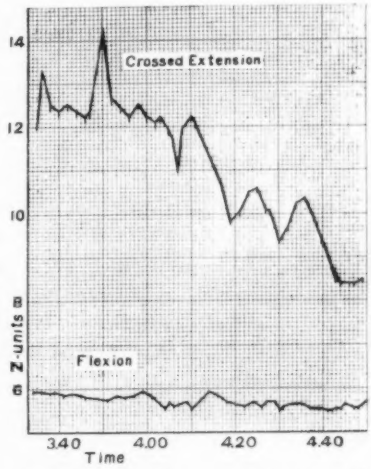


Fig. 6. Simultaneous variations of reflex threshold in flexion and crossed-extension reflexes (Experiment IX). Crossed-extension markedly less constant in threshold than flexion.

The comparative smoothness of the reflex curve is brought out when it is compared with a similar curve of threshold changes of the crossed-extension reflex; figure 6 is a record of the simultaneous behavior of the two reflexes, flexion being recorded as already described, crossed extension by the contraction of the quadriceps group of muscles, the patellar tendon being attached to a myograph lever and the femur held firmly by a pin passing through its lower end and into a block of wood; flexion varies in threshold from 5.50 to 5.95 Z units, crossed extension from 8.40 Z units to 14.30 Z units. The semi-rhythmic waves of variation in crossed-extension threshold with their crests at 4.10, 4.25 and 4.36 have been noted in a similar record from another animal. The smoothest flexion reflex curve obtained, was that of experiment VI (fig. 5), where the threshold for forty-five minutes showed a total variation of only 0.4 Z unit; the greatest change in any single minute was 0.3 Z unit (at 2.36, fig. 5) so that the total alteration was approximately 1.3 of this maximal single change.

[15] Gruber: This Journal, 1913, xxxii, 221.

The central connections between the neurones of the arc, that is, the synapses, have been held responsible for the greater variations in threshold of the reflex as compared with the nerve-muscle. This does not mean that any given synapse shows changes in resistance to the passage of the nerve impulse, corresponding to the undulations of a reflex curve such as that of figure 2; such a curve is not incompatible with the supposition that each synapse is obeying the "all-or-none" law. If we postulate a sufficient number of independent arcs involved in the reflex contraction of the tibialis anticus, each with its own synaptic resistance and each following the "all-or-none" law, we may explain all the observed variations in threshold; the complete dropping out of a group of synapses with low resistance will raise the threshold for the entire muscle to a new level, and their return to function will lower it.

SUMMARY

1. A liquid electrode (modified from Lucas) is described, by which mammalian nerve in situ may be stimulated for periods of several hours, under more uniform conditions at the point of stimulation than is possible with the platinum wire form of electrode.

2. The difference in threshold between successive readings one minute apart, may be from 1.6 to 7.6 times as great for the flexion reflex, (tibialis anticus muscle), as for the nerve-muscle preparation, the same muscle being used in each case.

3. The curve of reflex threshold variations changes its direction more frequently than the nerve-muscle curve for equal lengths of time; that is, the nerve-muscle curve is the smoother.

4. The nerve-muscle threshold has been observed to alter as much as 2 Z units (roughly ten times the maximum minute-to-minute alterations) in the course of an hour. It is the rule for such a change to be gradual, but an exception is described.

5. The nerve-muscle threshold showing least alteration remained for thirty-six minutes without exceeding in total change 0.16 Z unit, or about three times the maximum change in any single minute.

6. The reflex threshold showing least alteration remained for forty-five minutes within the limits of 0.4 Z unit, or 1.3 times the maximum change in any minute.

7. The threshold for crossed-extension varies more widely than the threshold for flexion. In the experiment described, the total variation

for crossed-extension was 5.90 Z units; for flexion, 0.45 Z unit, during the same period of time.

I am indebted to my wife, Helen Nichols Porter, for much assistance in this investigation.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 68.

THE BEHAVIOR OF HOLOTHURIANS IN BALANCED ILLUMINATION

W. J. CROZIER

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I. The behavior of phototropic animals when so situated as to be illuminated from opposite directions gives important information regarding the nature of the mechanism of stimulation. It was held by Loeb (1) that a phototropic organism, when placed at the center of the line joining two equal sources of illumination, should move in a direction perpendicular to this line. The accuracy with which this kind of response is obtainable in a suitable organism is demonstrated by Patten's (2) experiments with the negatively phototropic larva of the blowfly. Patten further found that the angular deflection of the larva's path from the perpendicular was so related to the percentage difference in intensity between the two lights, when these intensities were caused to alter in a graded series of ratios, as to produce a smooth curve when these values were plotted on the axis of ordinates and abscissae, respectively. On the basis of this graph, and taking into account the blowfly larva's method of locomotion, Patten was able to show (3) that the responses appear to be determined by the presence of two photosensitive surfaces inclined to each other at a definite angle. The definite path of progression adopted by the larva under the influence of two opposed unequal beams may be predicted on the assumption that the animal ceases to deflect from its original locomotor path—which in these experiments is a straight line normal to that connecting the sources of light—as soon as the luminous intensity on these mutually inclined surfaces is made equal by the larva's position.

II. There may be deduced from this principle the corollary that, if the photoreceptive surfaces of an animal are so arranged as to be sensibly *parallel*, no definite position should be assumed by this organism when bilaterally illuminated. For, in such a case, if the balanced lights were *equal* in intensity, it would be impossible for the animal to place itself in a position which would result in unequal illumination of the two sides; while, if the lights were *unequal*, the amount of light received by

either side could not in any position be made equal to that incident upon the other. It is assumed, of course, that the longitudinal axis of the animal remains straight when orientation is completed.

Thus an animal of this kind might be clearly phototropic according to other criteria, and yet fail to satisfy the requirement of orientation under bilateral illumination which Loeb's contention demands. The existence of such organisms, however, would add further support to the assumption made by Patten, namely, that the exhibition of a definite path of locomotion under these conditions depends upon the final equality of illumination on each of the two photosensitive surfaces.

III. Certain pedate holothurians have been found to be different from most other echinoderms, in that they exhibit a pronounced bilaterality in their structure, coupled with a fixed direction of progression (4), (5), (6). Typical tropistic behavior is thus made possible. It has been shown that some of these holothurians (Holothuria surinamensis, H. captiva) are photo-negative, that they are sensitive to light over their whole surface, that they give well defined reactions to shading, and are non-reactive to a sudden increase in light intensity, yet orient away from the light with conspicuous precision. It is impossible to conceive, as I have already pointed out (4), that these animals are oriented by light in any way other than through the direct action of illumination upon their integument.

One of the holothurians referred to, H. captiva, is the most outstandingly bilateral of all the species which I have studied. Its lateral surfaces are so nearly straight and parallel that, to all intents and purposes, they may be regarded as strictly so; that is, longitudinal elements of its surface are parallel. I have previously described the accuracy with which it orients away from the light. Experiments were subsequently made to determine the behavior of this species when illuminated from opposite sides.

Inasmuch as the whole surface of H. captiva is sensitive to light, the higher sensitivity of the oral end should not greatly obscure the interpretation of such experiments. When horizontal light is employed, the sides of the animal may be regarded as equivalent to two photosensitive areas. This particular species of holothurian, fortunately, tends to preserve a straight position of the body.

In my present study upon this animal, the tests were made in a dark room, the animals being placed in a flat-sided aquarium with their long axes perpendicular to the line connecting the two sources of light. The lights used were two tungsten incandescent filaments, placed at

various distances from the holothurian in different experiments. In some cases the animal was induced to begin crawling in a direction perpendicular to that of the light beams before the latter were turned on. The intensity of the lights was varied by using filaments of different wattage (25 to 100 watts). Since the characteristic features of the results soon became evident in these experiments, no great refinement was necessary in the physical precision of the light adjustments.

IV. In no case did Holothuria captiva assume a path of locomotion definitely related to the intensities of the acting lights. In no case was it constrained to move, after the fashion of the blowfly larva, along a path inclined to the direction of the beams. A number of animals were found to move first toward one light, then toward the other; this was the case when the lights were of nearly equal intensity. Many individuals circled in an aimless, indeterminate way (as when illuminated from above), while others became curled into the shape of the letter S, the oral end, and frequently the cloacal end as well, being bent away from the stronger light.

When the intensity of the stronger illumination was made eight or nine times that of the weaker one, the holothurians almost invariably oriented precisely and away from the stronger light. But when they had moved somewhat toward the weaker light, their movements became quite irregular.

To meet the criticism that possibly the incandescent filaments as sources of light were too small as compared with the size of the animals, the latter being 6 to 8 cm. in length, experiments were tried with sunlight reflected from two mirrors, each measuring 15 by 20 cm. The holothurians were in a dark chamber, an opening on either side (10 by 8 cm.) allowing the sunlight reflected from the mirrors to enter the box from opposite directions. One or several lightly smoked glasses were then interposed between one mirror and the corresponding opening in the dark chamber. The behavior of the holothurians under these conditions was qualitatively identical with that in the trials with electric lights. Hence I conclude that H. captiva does not maintain a predictable path of locomotion under the influence of bilateral illumination.

V. The validity with which this result may be applied to the criticism of photic stimulation theories depends largely on the share taken by the general surface of the animal in photoreception. That this share is a considerable one, is shown by the following:

a. Excised pieces of the "dorsal" integument (including skin and muscle layers) which have been cut from the midbody surface of H.

surinamensis or H. captiva exhibit local wrinkling contractions when a spot of intense light is thrown upon them. The wrinkling movements cease when the strong light is removed. When first prepared, the pieces of the body wall are strongly contracted; but if they be allowed to lie in seawater for half an hour or so, or if they be subjected to gentle traction, they become somewhat relaxed, although the cut edges remain curled. It was on such relaxed preparations that the light test was made. Tube feet on isolated portions of the "ventral" surface also bend away from the light.

b. A phenomenon closely akin to "circus movements," and probably in principle identical with them, appears when these holothurians are exposed to general illumination from above, provided one side of the animal's surface has been rendered insensitive to light. In the narcotizing, cocaine seems to give the best results, but chloretone was also used. In the case of either narcotic, a strong solution (in seawater) was employed and the holothurian, while being held out of water, had one side painted over with the solution several times. The parts so treated soon become quite insensitive to light, while the untreated side is still sensitive, and its tube feet all remain normally functional. On being returned to seawater the holothurians soon assume a curved attitude, the narcotised side being the relaxed one (convex). If the animals are then strongly illuminated from above, they rapidly become curved in the opposite direction, the narcotised side being then the inner, contracted (concave) portion of the bend. Thus the holothurian bends toward the *unstimulated* side, whether the non-stimulation is determined by the relative absence of light, or by the enforced incapacity of its photoreceptors.

VI. These experiments afford evidence: (1) that the amount of light received by the photosensitive surface of Holothuria surinamensis and H. captiva determines their behavior in an illuminated field; (2) that the assumption of a definite path of progression, with respect to balanced illumination, depends on the non-parallel relation of photosensitive surfaces.

BIBLIOGRAPHY

(1) Loeb: Studies in general physiology, Chicago, 1905, Part I, 13.
(2) Patten: Journ. Exper. Zool., 1914, xvii, 213.
(3) Patten: This Journal, 1915, xxxviii, 313.
(4) Crozier: This Journal, 1914, xxxvi, 8.
(5) Crozier: Zool. Jahrb., Abt. Physiol., 1915, xxxv, 233.
(6) Crozier: Sci., N. S., 1916, xliii, 148.

THE EFFECT OF DEXTROSE GIVEN INTRAVENOUSLY ON BLOOD COMPOSITION AND URINARY SECRETION[1]

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In a previous paper (1) the writer has summarized the data at hand concerning the relations between the composition of the blood and diuresis. In addition to studies directed toward a broad and general explanation of these relations, the attempt has frequently been made to concentrate attention upon some single substance and its behavior in such a connection. It is desired to present here an addition to these studies in the form of a series of injections of different dextrose solutions, using the same animal throughout.

Only a few previous studies will be mentioned. Lamy and Meyer (2) could find no relation between sugar diuresis and systemic blood pressure, the viscosity, molecular concentration (as measured by the depression of the freezing point), or dry weight of the blood, or the volume of the kidney in chloralized dogs with oncometers in place. The diuresis according to them may begin at a time when the viscosity of the blood is unchanged or even increased. It is noted that in general the polyuria runs parallel to the concentration of sugar in the blood, but there are numerous exceptions to this rule. These exceptions usually take the form of a high blood sugar without diuresis.

Fisher and Wishart (3) working in Lusk's laboratory, find that after the administration of dextrose per os, the hemoglobin of the blood is much decreased, but has returned half way to normal before diuresis begins. The hemoglobin concentration is taken as a measure of blood volume, although its accuracy in this rôle is open to some question (4).

Ewing (5) determined the specific gravity of the blood, confirming Fisher and Wishart's observations for glucose administered intra-

[1] Read in abstract before the Society for Experimental Pathology, New York, December 29, 1916.

venously. He adds that diuresis may occur without change in the concentration (specific gravity) of the blood, or may be absent when the blood shows a well defined dilution. Diuresis is usually dependent on blood sugar level, but one experiment is a notable exception to this rule. When the blood sugar exceeds 0.4 per cent, the urinary sugar concentration appears to remain fairly constant, while as the blood sugar falls and diuresis decreases, the urinary concentration goes up. Ewing's injections were made rapidly.

Epstein (6) studied the relation of glycosuria to blood sugar and concluded that it depends on an increase in the total amount of sugar in the circulation, which must be determined by noting simultaneously the blood sugar concentration and the blood volume (as indicated by its hemoglobin content). When a glycosuria is precipitated by a hyperglycemia in this sense, the urinary sugar concentration must show a tendency to remain constant, since "diuresis in diabetes mellitus plays an important rôle in determining the total amount of sugar eliminated in the urine, but has no influence on its concentration or percentage." This relation is found to be so constant that Epstein thinks that there is a glycosuric constant, like that of Ambard for urea, which is higher in individuals with nephritic kidneys. In such cases a greater hyperglycemia must be present to produce the same glycosuria.

Arrous (7) working with intravenous injections, contrived a coefficient expressing the relations of the volume of fluid injected and the volume of urine excreted, which remained constant for each concentration of dextrose, regardless of the size of the dose.

Blumenthal (8) brought forward very clearly the necessity for including time in the conception of glucose tolerance and utilization, and Woodyatt (9) with his method of precisely timed injections made possible a more accurate study of the questions above outlined.

It was planned, therefore, to have the present work consist of two series of injections, using the same animal for all, that each injection might be better compared with the others. In the first series, the same quantity of glucose, per kilogram-hour, was to be administered each time, but dissolved in varying amounts of water, while in the second, the same amount of water per kilogram-hour was to be injected, but containing varying quantities of glucose. In each experiment the volume of the urine and the sugar of the blood and urine were to be followed.

The general outline of experimental technique was the same as that described in a previous paper (1). No anesthetic of any kind

was used. Blood was drawn from the jugular with pipet and needle. This procedure caused in the animal, which was of a very quiet and phlegmatic disposition, no perceptible disturbance, and the blood sugar curves give no evidence of fluctuations from this cause. Urine was removed by the suction catheter which has been described. The injections were made with a small motor-driven pump embodying the same principles as that of Woodyatt (10). The glucose used was purified by several recrystallizations from crude dextrose. For this product the writer is deeply indebted to Dr. George Peirce (formerly of this laboratory). The blood sugar determinations were according to the method of Lewis and Benedict. The heating was done in an autoclave. The urinary sugar was estimated by means of the polarimeter. The use of small tubes made it necessary to dilute only the very small samples. The urine was cleared with alumina cream, which gave a beautifully clear supernatant fluid. It was found however that there was a slight adsorption of sugar by the aluminium hydroxide; from 1 to 5 per cent, according to the concentration. Corrections were made for this adsorption.

TABLE 1

DEXTROSE GRAMS PER KILOGRAM—HOUR	WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
	40.0	20.0	10.0	2.5
	per cent	*per cent*	*per cent*	*per cent*
0.7		3.5		
1.0		5		
2.0	5	10	20	80
3.0		15		

Table 1 gives an outline of the injections made, with the quantities of water and sugar administered, per kilogram-hour. The percentage concentration of the injection fluid in each case is shown by the figures in the boxes, each figure representing an experiment actually performed. The duration of the injections varied slightly, but all ran four hours or more except the 0.7 gram to 20 cc. experiment, and in the comparative figures all have been calculated merely for the first four hours, except this experiment. The injection referred to lasted three hours, and is included merely for reference, since a different dog was used for it.

It is felt that presentation in the form of curves will be the most advantageous to the reader for grouping quickly the results. These

curves have all been drawn to the same scale. The abscissae represent time, the ordinates the amounts of the various substances. The solid line represents the volume of urine secreted, calculated as a rate, i.e., cubic centimeters per fifteen minutes. The dotted line shows the blood sugar in per cent. The interrupted (dash) line gives the concentration of sugar in the urine, again as per cent, while the dash and dot line combines the first and third as the rate of excretion, or grams per fifteen minutes for the sugar. The vertical solid line represents the end of the injection.

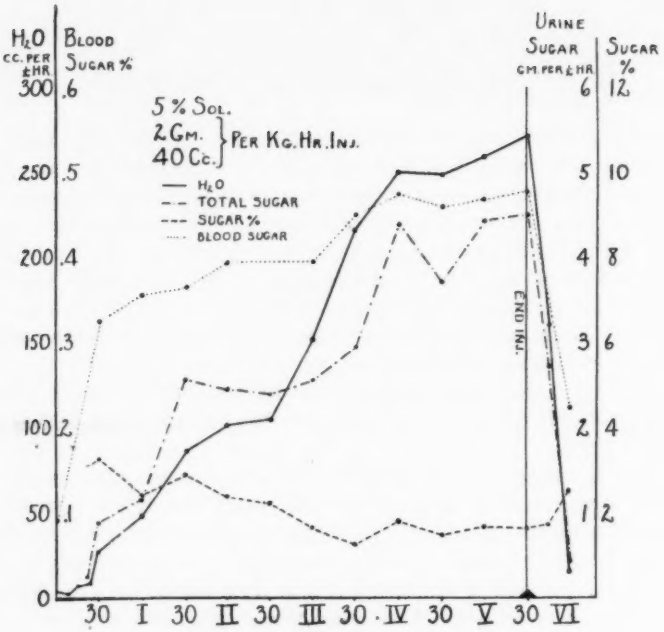
Some of the features of these injections may be best displayed by bringing together the curves from a number of experiments into composite charts.

Figure 8, the first of these charts, has four curves representing the water output rates in the experiments where the sugar injected remained constant. Each is labeled according to the concentration of the injection fluid used. As is to be expected, the more dilute solutions produce a greater diuresis, but that from the 80 per cent solution is practically as great as that from the 20 per cent solution. The diuresis begins in each case at the same time, namely about twenty to twenty-five minutes after the beginning of the injections.

Figure 9, the second composite chart, gives the rates of sugar output in the same four experiments. Here it is seen that, although the sugar intake is exactly the same per kilogram-hour in each case, the injection of 5 per cent solution, accompanied by a great diuresis, leads to a much smaller excretion of sugar than that of an 80 per cent solution, accompanied by very little diuresis. The other concentrations fit in well between. It must however be observed that this smaller excretion, in the case of the dilute injection, is principally a delayed excretion, since while with the 80 per cent injection, the sugar output shoots up most rapidly, it has, at about the fifth hour, atttained much the same level with all four concentrations of the injection.

This delay in excretion means, naturally, that there has been greater retention in the body of dextrose with the dilute than with the concentrated injections. The extent of this retention is shown numerically in table 2 in grams per kilogram-hour, for a four-hour period of injection.

This makes it seem that a copious diuresis interferes with the excretion of glucose by the kidneys, but it is probable that the true explanation is somewhat different. Zuntz (11) in metabolism experiments designed to show the protein sparing action of carbohydrate noticed


H₂O
CC. PER ½ HR.
BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR.
SUGAR %
5 % SOL.
2 GM.
40 CC.
PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300
250
200
150
100
50
0
.6
.5
.4
.3
.2
.1
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12
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6
4
2
30 I 30 II 30 III 30 IV 30 V 30 VI

Fig. 1

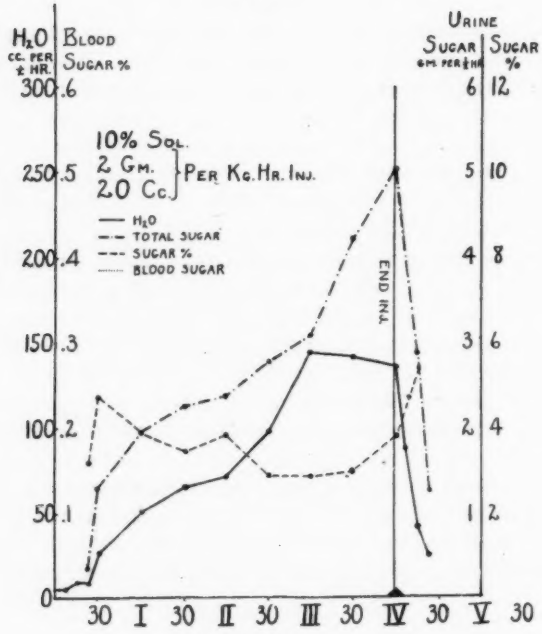
H₂O
CC. PER ½ HR.
BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR.
SUGAR %
10% SOL.
2 GM.
2.0 CC.
PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300
250
200
150
100
50
0
.6
.5
.4
.3
.2
.1
6
5
4
3
2
1
12
10
8
6
4
2
30 I 30 II 30 III 30 IV 30 V 30

Fig. 2

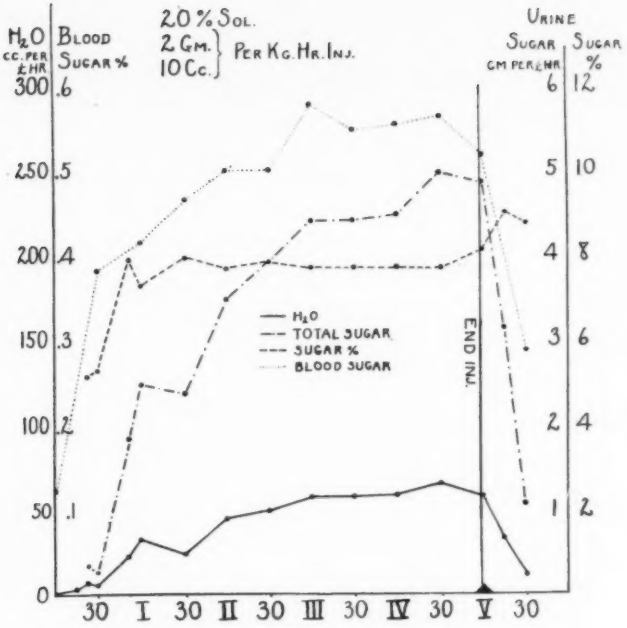
H₂O CC. PER ¼ HR.
BLOOD SUGAR %
2.0 % SOL.
2 GM. } PER KG. HR. INJ.
10 CC.
URINE SUGAR GM. PER ¼ HR.
SUGAR %
300
250
200
150
100
50
0
.6
.5
.4
.3
.2
.1
6
5
4
3
2
1
12
10
8
6
4
2
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
30 I 30 II 30 III 30 IV 30 V 30

Fig. 3

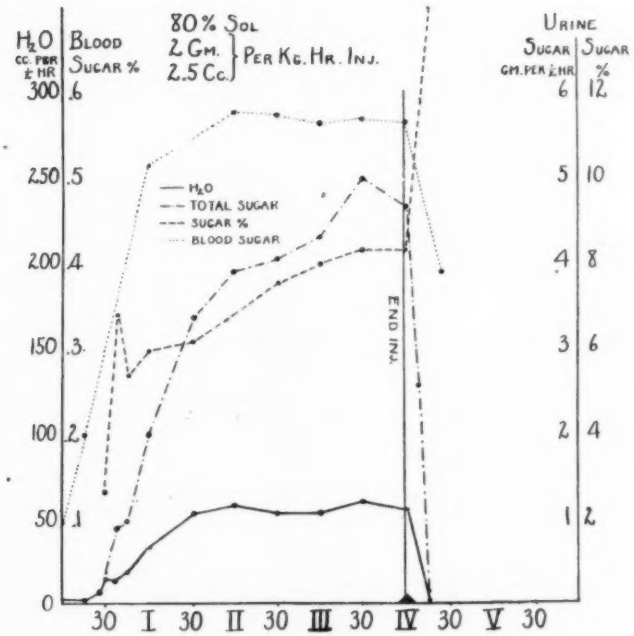
H₂O CC. PER ¼ HR.
BLOOD SUGAR %
8.0 % SOL
2 GM. } PER KG. HR. INJ.
2.5 CC.
URINE SUGAR GM. PER ¼ HR.
SUGAR %
300
250
200
150
100
50
0
.6
.5
.4
.3
.2
.1
6
5
4
3
2
1
12
10
8
6
4
2
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
30 I 30 II 30 III 30 IV 30 V 30

Fig. 4

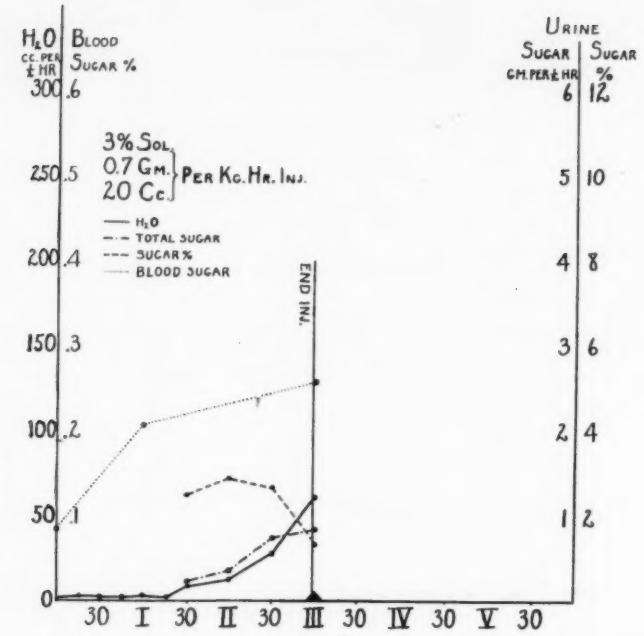
H₂O CC. PER ½ HR
BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR
URINE SUGAR %
3% SOL. 0.7 GM. 2.0 CC. PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300 250 200 150 100 50 0
.6 .5 .4 .3 .2 .1
6 5 4 3 2 1
12 10 8 6 4 2
30 I 30 II 30 III 30 IV 30 V 30

Fig. 5

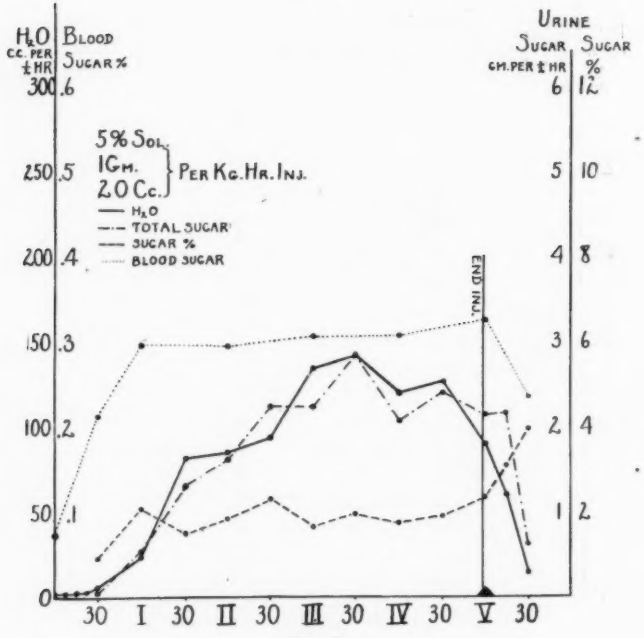
H₂O CC. PER ½ HR
BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR
URINE SUGAR %
5% SOL. 1 GM. 2.0 CC. PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300 250 200 150 100 50 0
.6 .5 .4 .3 .2 .1
6 5 4 3 2 1
12 10 8 6 4 2
30 I 30 II 30 III 30 IV 30 V 30

Fig. 6

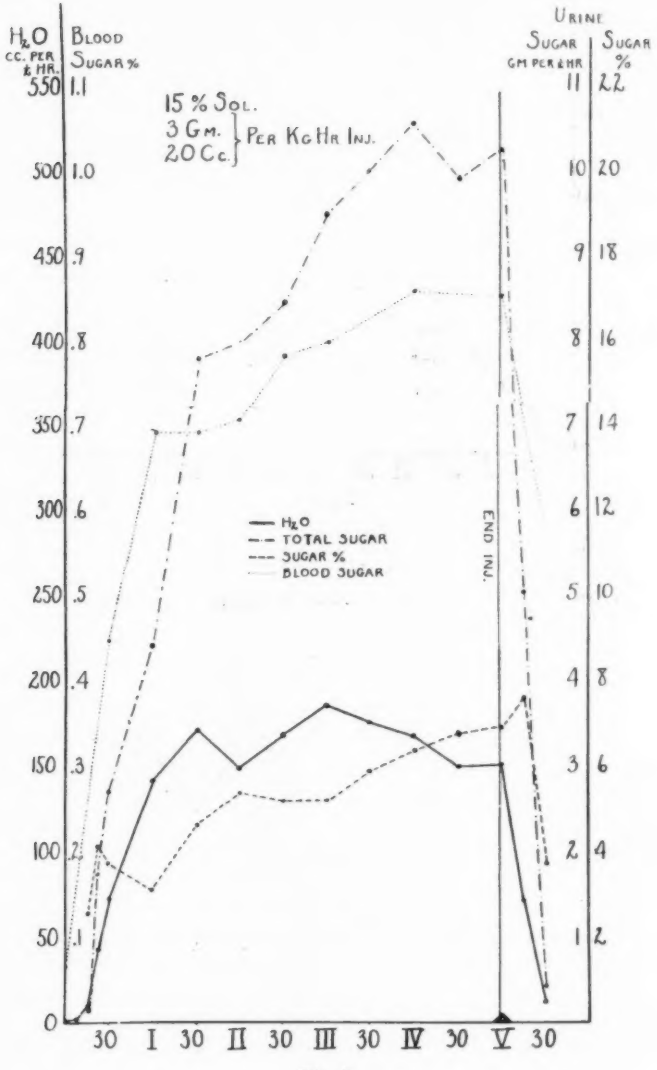
H₂O CC. PER ½ HR.
BLOOD SUGAR %
URINE SUGAR GM PER ½ HR.
SUGAR %
15 % SOL.
3 GM.
20 CC.
PER KG HR INJ.
END INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
550 500 450 400 350 300 250 200 150 100 50 0
1.1 1.0 .9 .8 .7 .6 .5 .4 .3 .2 .1
11 10 9 8 7 6 5 4 3 2 1
22 20 18 16 14 12 10 8 6 4 2
30 I 30 II 30 III 30 IV 30 V 30

Fig. 7

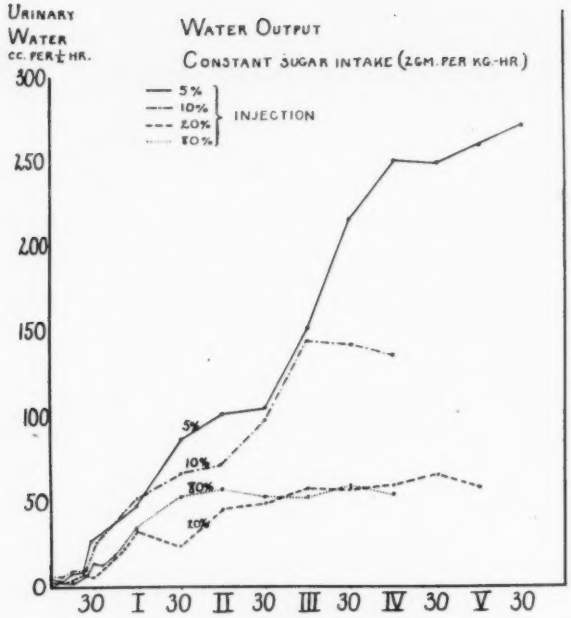
URINARY
WATER
CC. PER ¼ HR.
WATER OUTPUT
CONSTANT SUGAR INTAKE (2GM. PER KG.-HR.)
5%
10%
20%
30%
INJECTION
300
250
200
150
100
50
0
30 I 30 II 30 III 30 IV 30 V 30

Fig. 8

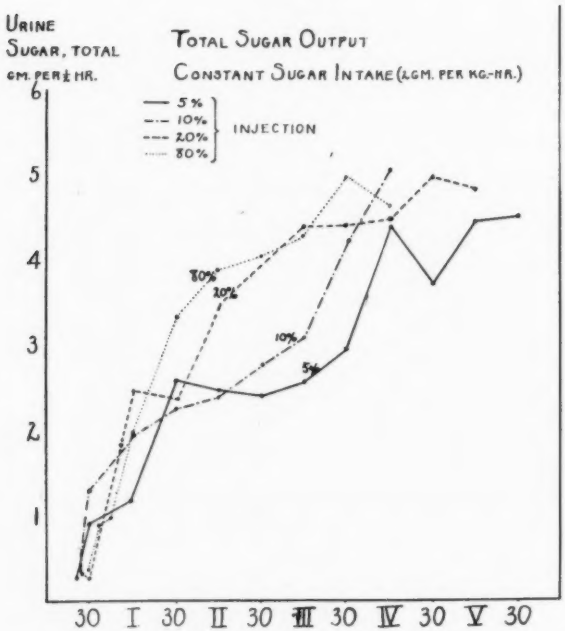
URINE
SUGAR, TOTAL
GM. PER ¼ HR.
TOTAL SUGAR OUTPUT
CONSTANT SUGAR INTAKE (2GM. PER KG.-HR.)
5%
10%
20%
30%
INJECTION
6
5
4
3
2
1
30 I 30 II 30 III 30 IV 30 V 30

Fig. 9

TABLE 2

DEXTROSE GRAMS PER KILOGRAM—HOUR		WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
		40	20	10	2.5
		Sugar grams per kilogram—hour	Sugar grams per kilogram—hour	Sugar grams per kilogram—hour	Sugar grams per kilogram—hour
2.0	Excreted	0.43	0.49	0.52	0.61
	Retained	1.57	1.51	1.42	1.39

that when, in his animals, carbohydrate was added to the diet, there was a sudden great increase in weight, due to a retention of water. This finding has been confirmed by Wimmer (12), Chauveau[2], Bischoff and Voit[2] and others. These experiments offer further striking confirmation by an entirely different method of investigation.

The following table (table 3) shows the fate of water injected with the sugar.

TABLE 3

SUGAR GRAM PER KILOGRAM—HOUR		WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
		40	20	10	2.5
		Water	Water	Water	Water
		cc.	*cc.*	*cc.*	*cc.*
2.0	Excreted	972	718.4	328	364
	Retained	788	178	94	−258

Although the diuresis was much greater with the dilute solutions, the amount of water retained was, like the sugar, also greater. The retention decreased steadily until, with the 80 per cent injection, there was an actual loss of water to the organism. Although one need not go as far as Zuntz in assigning a numerical value to the relation between the amount of water necessary for glycogen formation and the glycogen formed, yet it is clear that the retention curves of sugar and water run parallel to each other in these experiments. What has occurred, then, is an increased storage of glucose in the body, and one must seek further to make clear the effect of dilution of the injection on kidney function considered separately.

The data from the blood sugar determinations throw additional light on the mechanism by which this result is accomplished. They

[2] Quoted by Allen: Glycosuria and diabetes.

appear in figure 10. No blood sugar figures are available from the 10 per cent injection. When the strong solution (80 per cent) is injected, the blood sugar rises very sharply, nearly to 0.6 per cent, in striking parallelism with the rate of total sugar output in the urine and then remains approximately constant. With the weak solution, however, the initial rise is only in the neighborhood of one-half as great to be followed by a leisurely but steady rise through the five and one-

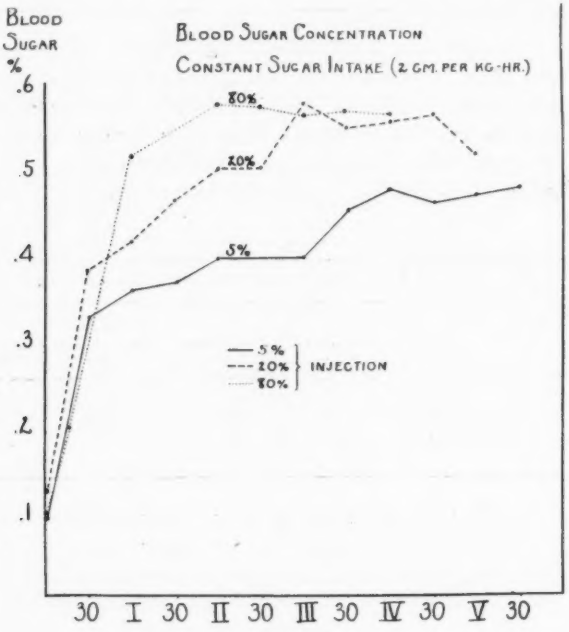

Fig. 10

half hours of the experiment. If figures for the water content of the blood were at hand, the interpretation of these findings would be assisted, but unfortunately they are not. The influence of dilution of the blood, with its attendant plethora, must be considered by indirect approach. This can be done after a glance at tables 2 and 3 and figure 10. In the 40 cc. (5 per cent) experiment, 788 cc. of water were retained, while a total of 18.7 grams sugar was excreted (see protocols). In the 2.5 cc. (80 per cent) experiment, 258 cc. of water were lost to the

body, the output being greater than the intake, and 26.6 grams sugar were excreted, the same quantity of sugar (88 grams) having been injected in each case. The animal then contained some 1046 cc. more of water and 7.9 grams *more* of sugar at the end of the first than at the end of the second experiment mentioned. The animal weighed about 11 kilograms, which would indicate a blood volume of roughly 770 cc. Now at the end of the 2.5 cc. (80 per cent) experiment, the blood sugar stood at 0.56 per cent, and even if one assumes—which is manifestly an exaggeration—that the entire 258 cc. which were lost during the experiment came from the blood and were not replaced, the circulation then contained $(770–258) \times 0.0056 = 2.87$ grams sugar, in 512 cc. of blood. Proceeding on the assumption that all of the 1046 cc. of fluid and 7.9 grams of sugar retained in excess in the 40 cc. (5 per cent) experiment remain in the plethoric blood, there would be in circulation at the end of this experiment $(1046 + 512 =)$ 1558 cc. of fluid containing $(7.9 + 2.87 =)$ 10.8 grams of sugar. If this were the case the concentration would be $(10.8 \div 1558 =)$ 0.69 per cent, but the actual observed concentration at the end of the experiment was 0.476 per cent, so that it is evident that at least part of the 7.9 grams retained in excess has gone to the tissues along with the bulk of the retained sugar. In addition, to assume that 1046 cc. of fluid remain in the circulation is going far beyond the necessities of the situation, since it would be an increase in blood volume of 136 per cent, and it is doubtful if this can occur.

In the experiments of Fisher and Wishart, it was calculated from the hemoglobin content that the blood volume might be increased by as much as 50 per cent after the ingestion of glucose. It is unfortunate that no accurate method of determining blood volume is available. Similar calculations for the 10 cc. experiment demonstrate the same facts, even more conclusively.

One can then conclude definitely that hydremia does not account for all of the sugar which is retained in the body in the dilute injection experiments in excess over that retained in the concentrated injection experiments, and that a considerable part of this sugar leaves the circulation for the tissues. This is equivalent to saying that the presence of a greater quantity of water in the injection fluid accelerates the storage or at least the taking over of dextrose by the tissues.

These considerations now enable one to return to the question of the relative diuretic efficiency of concentrated and dilute solutions. If it is true that the presence of a plentiful excess of water assists the

flow of both sugar and water from the vessels to the tissues, what effect has it on the flow of the same substances from the blood into the urine?

Sugar in the body can scarcely affect the kidney except as it comes in contact with the renal cells while circulating in the blood. To make a fair comparison, therefore, times should be chosen during the various experiments at which equal concentrations of sugar in the blood occurred.

Since more sugar has been removed from the circulation by other means in the case of the dilute injections, the kidney, having to deal with a blood of lower glucose concentration, does not receive as great a stimulus as in the case of the concentrated injections until much later in the experiment. If, for instance, the even figures for blood sugar level of 0.1 per cent, 0.2 per cent, 0.3 per cent, etc., are arbitrarily chosen, and the rate of sugar excretion noted in each experiment at the time each of these levels was reached, a table like the following rough example (table 4) can be made.

TABLE 4

BLOOD SUGAR PER CENT	WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
	40	20	10	2.5
	Sugar gram per 15 minutes	Sugar gram per 15 minutes	Sugar gram per 15 minutes	Sugar gram per 15 minutes
0.1	0		0	0
0.2	0		0	0
0.3				
0.4	2.75		1.2	1.3
0.5			3.0	2.25
0.6			4.5	3.75

Glycosuria begins in each experiment when the blood sugar is between 0.2 and 0.3 per cent. At 0.4 per cent the 40 cc. injection experiment is excreting 2.75 grams of sugar per fifteen minutes, while much less is being put out in the case of the more concentrated injections. From this standpoint, then, at a given level of blood sugar, the presence of an excess of water accelerates the passage of sugar from the blood into the urine, just as it does from the blood to the tissues.

Comparing figure 10 and figure 9, it will be seen that the nearest approach to parallelism exists between blood sugar and total sugar

output. This relation is maintained in all the experiments, the total sugar curve showing a tendency to hold its course more or less regardless of changes in the quantity of urine, especially the minor ones, which are compensated for in a truly remarkable way by changes in the concentration. This is represented graphically in some of the curves—the concentration changing one way, the quantity the other, and the total sugar line continuing its original course.

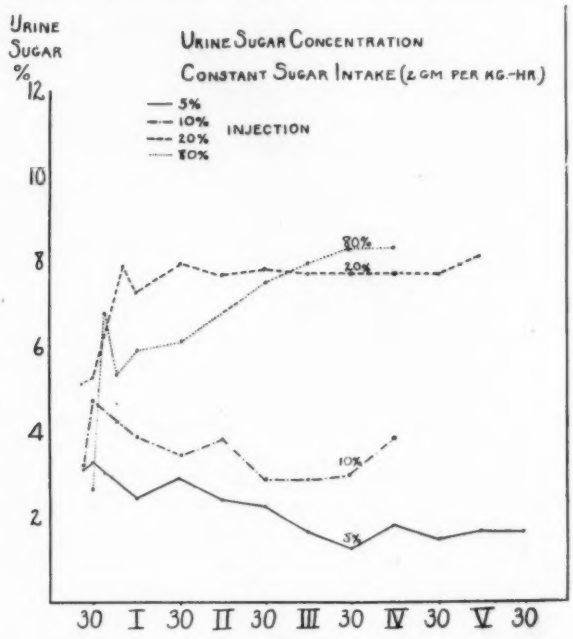

Fig. 11

Figure 11 represents the urinary sugar concentration in the first four experiments. In those cases with copious diuresis the concentration falls off as the diuresis increases. The concentration in the 80 per cent (2.5 cc.) injection is below that in the 20 per cent (10 cc.) injection at first, because the diuresis commenced earlier with the 80 per cent injection. The concentration does not seem to run parallel with the blood sugar, but varies inversely as the quantity of water available. Thus in figure 7 the steady increase in the sugar output

is obtained by a steady increase in the concentration, the urine quantity remaining constant, while in figure 3 exactly the reverse is true. Epstein's (6) statement, as noted above for diabetics, that the concentration is constant, the sugar output varying with the quantity of urine excreted, is certainly not true for a normal dog excreting sugar under these conditions. The concentration may continue to rise when the blood sugar goes above 0.4 per cent (fig. 7) contrary to the statement of Ewing (5).

The series of injections in which the amount of water given remained constant (20 cc. per kilogram-hour), figures 5, 6, 2 and 7, shows that the water output in such a case is entirely dependent on the sugar content. This is in accordance with the well known fact that distilled water given intravenously causes no diuresis.

SUMMARY

It is felt that the experimental conditions described are unusually free from disturbing influences.

Under these conditions the sugar output per unit of time shows a direct relationship with the blood sugar level.

Upon intravenous injection of a given quantity of dextrose, the amount of diuresis produced depends on the amount of water available, within certain limits. If a very concentrated solution is given, the body is robbed of water.

If large quantities of water are given with the dextrose, its percentage retention in the body is increased and water is retained along with it, so that less sugar is excreted, despite diuresis.

This retention is not confined to the blood, but occurs also in the tissues.

If more water is available, more dextrose is excreted into the urine *at a given level of blood sugar* than when less water is available.

I am much indebted to Mr. J. H. Janney of the fourth year class in the medical school for his valuable assistance in carrying out these experiments.

BIBLIOGRAPHY

(1) DAVIS: Journ. of Urol., 1917, i, 113.
(2) LAMY AND MEYER: Journ. de Physiol. et Pathol. Gen., 1904, vi, 1067.
(3) FISHER AND WISHART: Journ. Biol. Chem., 1912, xiii, 27.
(4) LAMSON: Proc. Natl. Acad. Sci., 1916, ii, 365.
(5) EWING: Proc. Soc. Exper. Biol. and Med., 1916, xiii, 69.
(6) EPSTEIN: Proc. Soc. Exper. Biol. and Med., 1916, xiii, 67.
(7) ARROUS: Compt. Rend. Soc. Biol., 1904, ii, 258; 1907, i, 649, 807, 845.
(8) BLUMENTHAL: Hofmeister's Beitr., 1905, vi, 329.
(9) WOODYATT: Journ. Amer. Med. Assoc., 1915, lxv, 2067.
(10) DAVIS AND GORTON: Journ. Urol., 1917, i, 136.
(11) ZUNTZ: Arch. f. Anat. v. Physiol. (Physiol. Abt.), 1898, 267; Biochem. Zeitschr., 1912, xliv, 290.
(12) WIMMER: Zeitschr. f. Biol., 1911, lvii, 185.

FURTHER STUDIES ON THE EFFECT OF ADRENALIN UPON MUSCULAR FATIGUE

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Muscular activity in conjunction with Addison's disease is extensively discussed in the older literature. Before experimental work was begun on the adrenal glands it was well known that marked muscular weakness was characteristic of Addison's disease. In 1892, Abelous, Langlois and Charrin (1) showed that persons suffering from this malady were less capable of doing muscular work, as measured by the Mosso Ergograph, than were individuals suffering from tuberculosis. When it was discovered that adrenalin excited the sympathetic nervous system, study of its effect upon the muscular system was for a time abandoned.

Recently, however, the study of the relation of adrenalin to muscular activity has been resumed. Three methods have been employed. They are, the older method, that of extirpating the glands, and the newer methods, those of splanchnic nerve stimulation causing the glands to secrete their adrenalin into the circulation and of injecting the adrenalin into the circulatory system or into the solution in which the muscle is contracting.

In 1892 Albenese (2) removed the glands from frogs and rabbits. Upon cutaneous stimulation, he observed that a stronger current was necessary to elicit a response after extirpation than before. That this increase was not due to the operation was shown by subjecting animals to more severe operations than decapsulation. He therefore concluded that the function of the adrenal glands was to destroy or to transform the toxic substances which, as a result of muscular or nervous work, are produced in the organism. His results were confirmed by Boinet (3) who reported that rats recently decapsulated were much more quickly exhausted in a revolving cage than were normal animals. A similar loss of muscular power was recorded by Biedl (4).

Founding his belief upon experiments done alone and with Langlois (5), Abelous (6) maintained that the suprarenal capsules, in frogs and guinea pigs, can modify, neutralize or destroy poisons which are produced in the course of muscular work or which accumulate in the organism after destruction of the adrenal glands. Adrenalin acts, he believed, as an antitoxin to fatigue. He observed that if the blood of an animal dying from decapsulation is injected into a normal animal true symptoms of fatigue result.

Oliver and Schäfer (7) in 1895 showed that adrenal extract has a bettering effect on the contraction of an unfatigued muscle. After injecting the extract subcutaneously into a frog and then excising the gastrocnemius muscle, they obtained a curve of contraction which was 33 per cent higher and 66 per cent longer than that of the corresponding muscle not supplied with the extract. A similar prolongation of the muscle curve was observed after the extract was injected intravenously into a dog. Analogous experiments were performed in 1915 by Kuno (8) and in 1916 by Takayasu (9), who obtained opposite results. Takayasu claims that adrenalin has a depressing rather than a bettering effect upon muscular contraction. He quotes Schäfer as saying of his own early experiments that he believed the results he obtained were due not to the action of adrenalin but to some impurities contained in the commercial extract which was employed. Schäfer also makes note of this belief in his recent publication, *The Endocrine Organs* (10). These results would seem to dispose of Joteyko's (11) arguments that adrenalin is a sarcoplasmic excitant.

Betterments in fatigued muscles after injections of adrenalin were observed by Boruttau (12) working on frogs and Dessy and Grandis (13) working on salamanders. Dessy and Grandis claim that the adrenal extract produces a beneficial effect on fatigued muscle either when injected subcutaneously or when added to the solution in which an isolated muscle is contracting. Cannon and Nice (14) were unable to confirm Dessy and Grandis' observations in frogs' muscles similarly exposed to the adrenal extract.

In a case of neurasthenia, Pantanetti (15) recorded a marked betterment in the total amount of work done after six subcutaneous injections of adrenalin.

The influence of fatigue upon the adrenalin content of the suprarenal glands in dogs was studied by Battelli and Boatta (16). In completely exhausted animals the adrenal content of the suprarenal capsules was decreased to less than one-sixth the normal amount. These

investigators believe that during fatigue adrenalin is set free to keep up the blood pressure which has a tendency to lower itself by dilation of the vessels in the active muscles. That the chromaffin system is thus exhausted by prolonged muscular effort was later confirmed by Carl (17) on strychninized frogs. Bernard and Bigart (18) made examinations of the histological structure of the adrenal gland before and after muscular fatigue. It was discovered that after prolonged muscular activity the gland contained numerous vacuoles. Bardier and Boone (19) confirmed them.

Carnot and Josserand (20) found that if 0.025 mgm. of adrenalin per kilo body weight was injected into the femoral vein of a normal dog there was an increase of 10 cm. of mercury in arterial pressure; if injected into the femoral artery (leg at rest) it produced an elevation in arterial pressure, of only 2 cm. of mercury. In one particular experiment upon a dog they injected 0.05 mgm. of adrenalin per kilo body weight into the femoral artery of a leg at rest and found that the blood pressure in the general circulation was increased 10 cm. of mercury. The opposite leg was then tetanized for fifteen minutes and at the end of that time 0.055 mgm. of adrenalin per kilo was injected into its femoral artery and the systemic blood pressure rose only 1.5 cm. of mercury. They claim that the adrenalin was neutralized or destroyed by the fatigue products.

Panella (21) observed a marked improvement with the use of adrenalin in the curve of contraction in heterothermic animals, frogs and toads, and also in homothermic animals, rabbits and guinea pigs, placed by special treatments (section of the bulb or profound narcosis), in a state of respiration, circulation and heat regulation somewhat like that of heterothermic animals. He believed that under normal mammalian conditions adrenalin has little effect because it is quickly oxidized or destroyed in the blood.

Radwánska (22) found that when the gastrocnemius muscle of a frog was wholly fatigued it could be made to contract again by injecting adrenal extract subcutaneously. He also found adrenin more effective in decapsulated than in normal frogs. Because the results were better when the muscle was stimulated through its nerve than when stimulated directly, be believed that adrenin acts upon the nerve endings. His belief was shared by Cannon and Nice (23) who thought the point of action of adrenin to be upon the nerve endings or neuromuscular junctions. They found that adrenin injected in small doses or secreted during splanchnic stimulation caused a marked improvement in the activity of the fatigued muscle.

A decrease in the irritability of the nerve in the nerve muscle of a frog as a result of the removal of adrenalin from the system by extirpation of the adrenal glands and a restoration of irritability upon injecting adrenalin into the same animal were demonstrated by Czubalski (24). He has assumed that the first phase of the action current is due to dis-assimilation or katabolism and the second phase due to assimilation or anabolism. Working upon this assumption he obtained evidence that the quick exhaustion of the muscles of decapsulated animals is due to slow and incomplete anabolism within the muscle. This process could be hastened by adrenalin.

In a series of papers published a few years ago, I (25) showed that adrenalin increased the height of muscular contraction when injected intravenously in small doses (0.2 to 0.5 cc. of a 1:100,000 solution), and that the same dose administered in the same manner would bring back to normal in five minutes or less the increased threshold stimulus caused by fatigue of the nerve-muscle or muscle, as would rest of one to three hours. I also showed by means of a comparative study of the effects of adrenalin and amyl nitrite that this improvement after fatigue was not due to a bettering of the circulation. Invariably the effect produced by adrenalin was greater although the effect upon the general blood pressure was the same. In some experiments the limb was perfused with warm Ringer's solution. If adrenalin was injected into this perfusion fluid a betterment in muscular contraction but always a decrease in the flow of the fluid (vasoconstriction) from the venous cannula was observed. The theory held by Radwánska that the action of adrenalin was on the nerve endings I disproved since I found that adrenalin has the same effect upon the muscles in which the nerve has been cut from nine to eighteen days as it has in normal muscles.

Recently Hoskins, Gunning and Berry (26) demonstrated that adrenin produces active vasodilation of muscle vessels and they believed that the betterment in the height of muscular contraction, which I demonstrated, was due, in part at least, to the betterment in circulation. In my experiments there had been three factors tending to bring about extreme dilation of the vessels in the active muscle. First, the nerves were severed and the vasomotor tonicity from the vasomotor center, therefore, inoperative; second, the rate of stimulation employed was favorable to dilation (27) and third, as Kaufmann (28) has shown, the fact that the muscles were active would presuppose actively dilated blood vessels. These circumstances led me to be-

lieve that in my former work any further dilation would have been so slight as to be negligible. Since I had not, however, separated the cutaneous circulation from the muscle circulation it seemed important that I repeat my work with the view to determining the condition of the blood flow through the muscle alone.

METHOD

In the earlier experiments the animals (cats) were anaesthetized with urethane (2 grams per kilo body weight by stomach), but later continuous ether anaesthesia was employed. By making a small slit through the skin on the outer side of either thigh, the anterior tibial nerve (peroneus communis) was isolated, cut and its distal end fastened in a Sherrington shielded electrode (29). The electrode was then held in place by fastening around it, with paper clips, the two flaps of skin.

Through another slit in the skin in the same leg the tendon of the tibialis anticus muscle was isolated from its insertion. It was then fastened to a muscle lever mounted upon a tripod base by a string passing about two pulleys. These pulleys were arranged so that the muscle pulled in its normal direction. One loop of cord about the hock and another around the foot just below the fastening of the tendon bound the leg to the board and made a very satisfactory nerve muscle preparation.

The strength of the stimulating current was 0.1 ampere in the primary circuit derived from a storage battery. The stimulating current in every case was a maximal break induction shock obtained from a glass knife blade key (30), which, propelled by a motor, made and broke the primary circuit. The rate ninety times per minute in these experiments was slow enough to produce vasodilation (27) in the vessels of the stimulated muscle. The muscle lever consisted of a piece of light straw 22 cm. in length from the axis to the tip of the parchment paper writing point. The tendon attached 3 cm. from the axis began at the moment of contraction to pull against an initial tension of 110 grams developed in a coiled spring. For each centimeter excursion of the muscle lever on the drum this was increased 5 grams. This spring was attached at the same position on the lever as was the muscle. Muscular contraction was, therefore, magnified about seven and one-third times.

The blood pressure, whenever recorded, was registered from one or the other carotid artery by means of a mercury manometer. A time marker which indicated intervals of five or of thirty seconds was placed at the atmospheric pressure line of the manometer. Thus, at any given muscular contraction, the height of blood pressure could be determined. Below the time marker was placed another signal magnet to indicate the time of the injection of adrenalin.

The rate of blood flow through the muscle was recorded on the kymograph paper in the early experiments by a simple key and signal magnet. Later an automatic drop recorder was substituted for the hand key. It consisted of a flat steel spring to which was soldered a platinum disc in contact with a platinum point adjustable by a thumb screw. The platinum point and steel spring were connected by copper wires to binding posts on the vulcanite arm which supported them. Each drop falling upon the spring broke the contact between the platinum disc and the point and the drop was recorded on the drum by the signal magnet. The recorded blood flowed through a cannula placed in the femoral vein. *All the branches* to the vein were tied off except the deep anterior tibial vein which comes from the tibialis anticus muscle and muscles of that region. The cutaneous vessels were tied and the limb was either skinned or mass-ligated above the hock. In the early experiments a necropsy was made after each series of observations to make sure that there had been a separation of the cutaneous and muscular circulations.

Usually adrenalin chloride but in many cases crystalline adrenalin in solution was injected into a cannula placed in the external jugular vein. The dilution of adrenalin with mammalian Ringer was made in all cases just after the operation so that the solution had no appreciable time for deterioration. This solution was kept at body temperature for injections.

Experiments were also performed in which the muscle was irrigated. The medium for irrigation, a warm (38.5 to 39.5°C.) Ringer's solution at a pressure of 60 to 70 cm. of water, ran through cannulae placed in the femoral artery and femoral vein after all the branches leading to and from these vessels were tied off except the two vessels of the deep anterior tibial region. The adrenalin, in most cases 1:100,000 but in some cases 1:1,000,000 and 1:1000 solution was injected into the running fluid close to the arterial cannula.

RESULTS

In no instance in my experiments did adrenalin in small doses (0.5 to 2 cc. of a 1:100,000 or 1:1,000,000 solution) produce dilation of the vessels in the limb in which the nerve was cut and stimulated at a rate favorable to dilation. In animals with the circulation undisturbed (except that the nerve was cut and stimulated) the vessels of the limb responded passively to adrenalin (0.5 to 2 cc. of a 1:100,000 solution) i.e., if the blood pressure decreased the rate of flow through the muscles decreased and vice versa. In the perfused limb with the nerve cut and stimulated vasoconstriction was obtained even with doses as small as 0.5 cc. of a 1:1,000,000 solution.

The action of adrenalin in intact muscles

Figures 1 and 2 are records made during one experiment and selected as typical of all the results obtained. The animal weighed 2.5 kgm. and was given 25 mgm. of hirudin intravenously as an anticoagulant just before the experiment and after the operation.

In figure 1 the blood pressure was 54 mm. of mercury. Upon injecting intravenously 0.5 cc. adrenin (1:100,000 solution) the pressure decreased to 44 mm. of mercury with a resultant decrease in the rate of blood flow through the muscle of 21 per cent and a concurrent increase in the height of muscular contraction of 55.5 per cent.

In other animals upon injecting 0.5 cc. of a 1:100,000 solution of adrenin no measurable change in the blood pressure but always an increase in the height of muscular contraction, in some cases as much as 35 per cent, occurred. The rate of blood flow remained unaffected. There is, of course, in all cases, a gradual decrease in rate and lowering of pressure due to the loss of blood from the recording vein.

Before figure 2 was recorded, 50 cc. of warm Ringer's solution was added to the blood that was lost by hemorrhage and the mixture was perfused back into the animal. As a result of the transfusion the blood pressure increased slightly (20 to 52 mm. of mercury). At *1* in figure 2, 2 cc. of a 1:100,000 solution was injected intravenously. The blood pressure rose 38 per cent and the height of muscular contraction was bettered 53 per cent. Since the same quantity of adrenin injected slowly at *2* produced no change in muscular contraction the betterment observed cannot be due to the injection of adrenin at *1* and *3*. Both the dilation of the vessels of the muscles and the increase in the height of muscular contraction were due to the increase

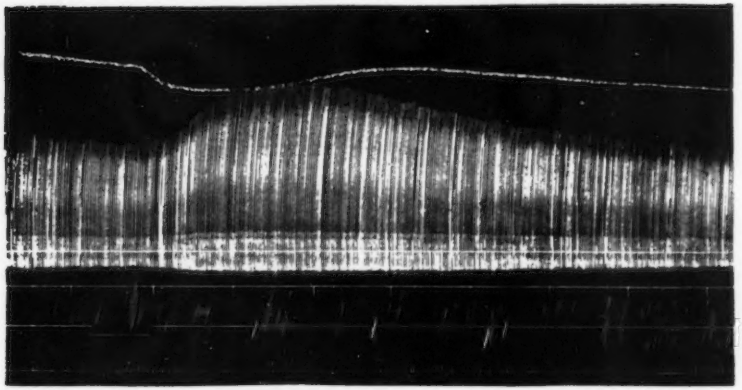

Fig. 1. In this and the following figure the upper curve is a record of blood pressure with mercury manometer, below it the record of muscular contraction. The lowest record indicates the number drops flowing from the venous cannula or limb circulation, above it is the signal record indicating the point of injection of adrenalin. Middle line time in thirty seconds. Hirudin was used as an anticoagulant. In this figure the time marker was placed 2 cm. below the atmospheric pressure level of the mercury manometer to allow room for muscular contraction. At the point indicated in the record 0.5 cc. of adrenalin (1:100,000) was injected.

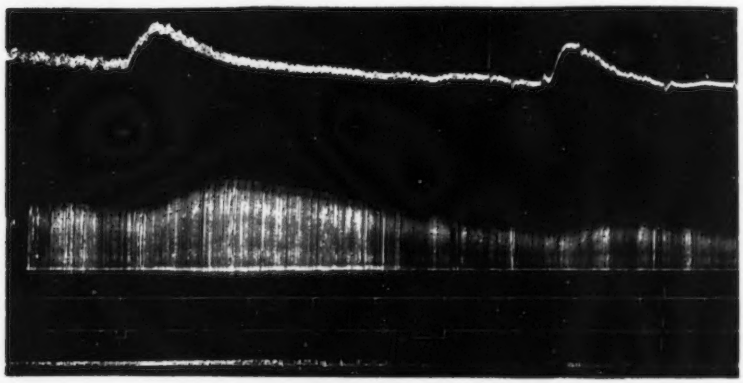

Fig. 2. Time marker 3.5 cm. below zero blood pressure. At the points indicated by the marker 2 cc. of adrenalin (1:100,000 solution) were injected.

in blood pressure. The increase in blood pressure is sufficient to account for the increased muscular efficiency (25).

The results obtained upon animals into which the lost blood plus Ringer's solution was perfused, were rarely satisfactory.

The action of adrenalin in perfused muscles

In the perfused muscles doses of adrenalin varying from 1 cc. of a 1:1,000,000 to 1 cc. of a 1:1000 solution produced a betterment in the height of muscular contraction with a decrease in the rate of flow of the perfusion fluid.

Figure 3 is a record obtained from an animal which had been given hirudin. Adrenin, 0.5 cc. (of a 1:100,000 solution) was injected at *1* and 2 cc. of the same solution was injected at *2*. As a result of the first injection there was a betterment of 17 per cent in the height of muscular contraction and from the second injection there was a betterment of 550 per cent with intermediary betterments, the whole lasting for six minutes. Here, as in most instances, the increase and subsequent decrease were gradual. After the first injection the rate of flow through the vessels decreased from seventy-four to thirty drops per thirty seconds and after the second injection the rate of flow decreased from thirty-five to less than one drop per thirty seconds. It is observed in this experiment as in many others that the maximal increase in the height of muscular contraction occurs simultaneously with the maximal constriction of the muscle vessels. These conditions are probably coincident and not interdependent.

When a muscle has ceased completely to respond to the original strength of stimulus it can be made to react again after an injection of adrenalin (see fig. 4). Here at *1*, 0.5 cc. of a 1:100,000 solution injected into the perfusion fluid produced a rise in the muscle curve of 26.6 per cent with no noticeable change in the circulation. At *2*, 1 cc. produced a rise of 32.5 per cent in muscular contraction and a decrease of 5 per cent in the rate of flow of the fluid through the muscle. At *3*, 1 cc. of the solution produced no noticeable effect upon the muscle. From point *3* to point *4* the muscle curve dropped until no contraction occurred. At *4*, 2 cc. of adrenalin were injected into the perfusion fluid. As soon as the adrenalin reached the blood vessels a marked vasoconstriction occurred, followed soon after by a slight muscular twitch which increased slowly until a contraction 6 mm. in height was obtained and then decreased to nil again. The length of time occupied by the entire recovery contractions was about six minutes.

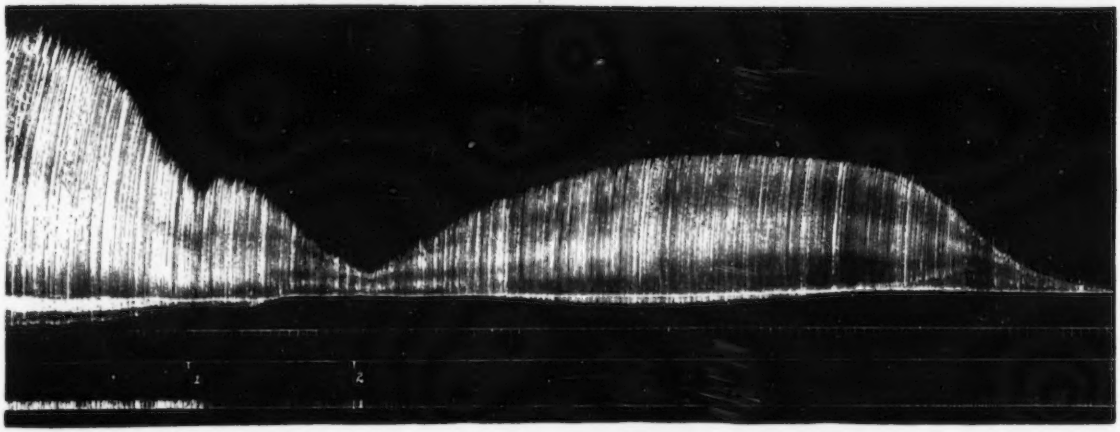

Fig. 3. Perfused muscle. At *1*, 0.5 cc. adrenalin (1:100,000 solution) was injected into the perfusion fluid. At *2*, 2 cc. were injected. Time in five seconds. The initial tension of the spring in this experiment was 140 grams with an increased tension of 7 grams per centimeter space on the drum. In this and the following figure the upper curve is the muscle record, below it the time record and the lowest line a record of the rate in drops of flow of the perfusion fluid through the muscle.

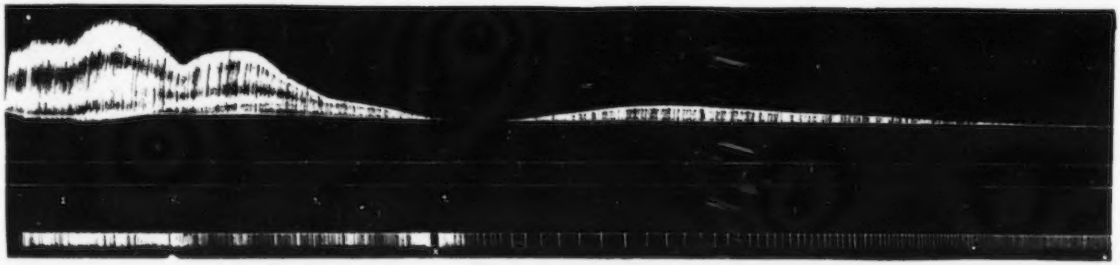

Fig. 4. A curve showing recovery of the muscular contraction in a completely fatigued perfused muscle. At *y*, the drum was stopped for five seconds. Adrenalin (1:100,000 solution) was injected, at *1*, 0.5 cc., at *2*, 1 cc., at *3*, 1 cc. and at *4*, 2 cc.; *x*, adjustment of the cannula. Time in thirty seconds.

Is adrenalin in large doses toxic?

Takayasu concluded that adrenalin in large doses was toxic in character and its effect resembled that of increased potassium salt concentration. Cannon and Gruber (31) remarked that in normal muscles adrenalin in large doses (1 cc. of a 1:10,000 solution intravenously) sufficient to cause a marked constriction of the arterioles, results in a lessened height of contraction and slowing or even a disappearance of the wavelike variations observed in rhythmically stimulated muscles. This muscular inefficiency they attributed to a deficient blood supply. The effect upon perfused muscles, of more concentrated solutions than that employed by Cannon and Gruber, is not different from that produced by more dilute solutions. Here may be given the results of one typical experiment. Adrenalin 0.5 cc. (1:100,000) was injected into the perfusion fluid. There resulted from this injection a betterment of 100 per cent in the height of muscular contraction. After this injection 0.5 cc. adrenalin (1:1000) was injected and there was observed a twenty-seven fold increase in the height of muscular contraction which required sixteen and one-half minutes for its development and return to the original height. Here as before marked vasoconstricton occurred.

The effect of adrenalin upon the make shock. In figure 4, the irritability was increased by adrenalin to such an extent that the make shock, which had been subminimal became submaximal at *1* and *2*. In two instances in which a 1:10,000 solution was employed the make shock contraction became equal to the break shock contraction.

In my experiments muscles exposed to the action of hirudin seemed to be more vigorous and to respond to adrenalin better than did normal muscles. According to Tatum (32) adrenalin has no action upon hirudin blood and hirudin plasma and vice versa. The question of the effect of anticoagulants upon muscular contraction is open for further investigation.

How is the specific effect of adrenalin in muscular fatigue shown?

All the curves presented in this paper except figures 2 and 4, *3*, show the specific effect of adrenalin on muscular fatigue. This effect is noted whether the limb is intact or perfused.

Figures 2 and 4, *3*, are given to show that at times adrenalin does not have this effect. Cannon and Nice (23) show a similar curve

(fig. 5, page 52) from a muscle of an animal undergoing splanchnic stimulation. In their experiment the blood pressure was kept at a fixed level by compression of the thorax. During that time, although there was undoubtedly a secretion of adrenalin caused by splanchnic stimulation, the muscle remained unaffected. At the moment at which the thorax was released, the blood pressure rose and with it the height of muscular contraction rose 466 per cent.

In an earlier article (33) I showed curves (figs. 6 and 7) in which adrenalin had the effect of lowering the blood pressure and the muscle curve 18.7 per cent in figure 6 and 17.3 per cent in figure 7. It might be argued in these cases that adrenalin may have had its specific effect upon the muscle but that it was obscured by the fall in blood pressure and decreased blood flow. Such is probably not the case as seen from *b* in both figures. An equal decrease in the height of muscular contraction and lowering of the blood pressure without the presence of adrenalin was brought about in figure 6, *b*, artificially by compressing the thorax. In figure 7 at *b* adrenalin was injected as at *a* but the blood pressure was maintained at a normal level by stimulating the splanchnic nerves with the adrenal glands tied off. The muscle curve was unaffected.

In the above described cases the only effect of adrenalin on the muscle curve must be a passive one. By lowering or raising the blood pressure it decreases or improves the muscle irritability.

The curves herein presented other than 2 and 4, *3*, show the specific effect of adrenalin. In figure 1 this is especially great for animals in which the circulation is intact. The injection of adrenalin brought about a decrease in the rate of blood flow but an increase in the height of muscular contraction of 55 per cent. In perfused limbs the increase is so great as to be almost unbelievable. Figure 3 shows an improvement of 550 per cent. In other experiments improvements of 2730 per cent were observed.

How does adrenalin produce its effect?

There has been much speculation as to the probable point of action of adrenalin. Although the question cannot be settled in this article it may not be amiss to discuss some of the possibilities.

1. *Does it act upon Langley's "receptive substance"?* Langley (34) has demonstrated a hypothetical "receptive substance" between the nerve endings and the muscle. In another paper (35) I showed that

the threshold stimulus of a denervated muscle was unaffected by curare and that adrenalin had no effect upon the curare threshold in this same muscle. I found, however, that the threshold of a curarized muscle with the nerve intact was affected by fatigue and that adrenalin counteracts this fatigue. If then curare acts upon the "receptive substance," fatigue and adrenalin must act at another point nearer the muscle than the "receptive substance."

2. *Is adrenalin a sarcoplasmic excitant?* It has been my observation that adrenalin does not lower the threshold of a normal, unfatigued muscle (25). Were adrenalin a sarcoplasmic excitant as suggested by Joteyko (11) it would probably do this and if it were an excitant at all it would certainly not be necessary to fatigue the muscle before adrenalin produced a marked result. This conclusion is further substantiated by Kuno and Takayasu.

3. *Does it neutralize, destroy or transform fatigue products?* One of the most reasonable explanations is this, that adrenalin has some effect upon lactic acid, rendering it less harmful to the active muscle. Various experimenters some of whom are Abelous and Langlois, Albanese, Carnot and Josserand, Joteyko and Gruber, have thought this a feasible explanation. This leads to the fourth possibility.

4. *Does adrenalin hasten the conversion of glycogen into sugar and does it assist in the reconversion of lactic acid into sugar?* It has been found that in a normal muscle with the circulation intact the same dose of adrenalin injected repeatedly brings about approximately the same reaction while in a perfused muscle a second or third injection has to be larger than the first to bring about the same response. This would seem to indicate that adrenalin acts upon some substance supplied through the blood or which is present in the muscle. Cannon and Nice (23) came to the conclusion that the increased muscular contraction cannot be due to hyperglycaemia because a typical rise could be obtained when the liver was removed. If an injection of adrenalin liberates sugar from the liver it does not seem improbable that the same injection could liberate sugar in the muscle. If that is the case, such liberation of sugar in the muscle might not be indicated in the blood and might along with the normal supply of sugar in the blood, supply the necessary energy for the increased muscular activity. This inference is in accordance with that of Cybalski (24) who believed that the decreased action current in the nerve muscles of decapsulated frogs and fatigued animals was due to the sluggish anabolic process and adrenalin is capable of accelerating this process,

but it is not in accordance with that of Wilenco (36) who maintains that the ability of the organism to burn sugar is decreased by adrenalin.

It must be admitted that none of these explanations is without its weak points and that none completely accounts for the various apparrently contradictory results obtained. At present a chemical study is being undertaken to determine more definitely the point of action of adrenalin.

SUMMARY

In the fatigued unaltered nerve muscle adrenalin may increase the height of muscular contraction by a twofold action, by improvement of the blood supply (vasodilation) and by its chemical action upon some substances in the muscle.

In a muscle in which the nerve is cut and stimulated, adrenalin in small doses, however administered, does not better the circulation and must therefore produce its effect of increasing the height of muscular contraction by its chemical (specific) action alone.

The following three processes which normally go on in the muscle may be greatly accelerated by adrenalin and it is not improbable that one or all of these will finally prove to be the way in which adrenalin produces its effects:

1. The conversion of glycogen into sugar.
2. The reconversion of lactic acid into sugar (transformation of fatigue products).
3. The oxidizing of lactic acid into carbon dioxide and water (destruction of fatigue products).

BIBLIOGRAPHY

(1) Abelous, Langlois and Charrin: Compt. rend. Soc. de Biol., 1892, xxiv, 623 and 721.
(2) Albanese: Arch. Ital. de Biol., 1892, xvii, 239.
(3) Boinet: Compt. rend. Soc. de Biol., 1895, 273, 325, 498 and 646.
(4) Biedl: Innere Sekretion, Berlin, 1910, 149.
(5) Abelous and Langlois: Arch. de Physiol., 1892, xxiv, 269 and 465.
(6) Abelous: Arch. de Physiol., 1893, xxv, 437 and 720; ibid, 1894, xxvi, 433.
(7) Oliver and Schäfer: Journ. Physiol., 1899, xviii, 230.
(8) Kuno: Journ. Physiol., 1915, xlix, 139.
(9) Takayasu: Quart. Journ. Exper. Physiol., 1916, ix, 347.
(10) Schäfer: The endocrine organs, New York, 1916, 65.
(11) Joteyko: Journ. Med. de Bruxelles, 1903, viii, 421.
(12) Boruttau: Arch. f. d. gesammt. Physiol., 1899, lxxviii, 109.
(13) Dessy and Grandis: Arch. Ital. de Biol., 1904, xli, 225.

(14) Cannon and Nice: This Journal, 1913, xxxii, 56.
(15) Pantanetti: Arch. Ital. de Biol., 1895, xxii, 17.
(16) Battelli and Boatta: Compt. rend. Soc. de Biol., 1902, liv, 1203.
(17) Carl: Deutsch. Med. Wochenschr., 1911, xxxvii, 1827–29.
(18) Bernard and Bigart: Compt. rend. Soc. de Biol., 1902, liv, 1400.
(19) Bardier and Bonne: Compt. rend. Soc. de Biol., 1903, 355.
(20) Carnot and Josserand: Compt. rend. Soc. de Biol., 1902, liv, 1472; ibid, 1903, lv, 51.
(21) Panella: Arch. Ital. de Biol., 1907, xlviii, 430.
(22) Radwánska: Anzeiger d. Akad., Krakau, 1910, 728; reviewed in Zentralbl. f. Biochem. u. Biophysik, 1911, xi, 467.
(23) Cannon and Nice: This Journal, 1911, xxix, 24; ibid, loc. cit., 49.
(24) Czubalski: Anzeiger d. Akad., Krakau, 1912, v, 470; ibid, 1913, 184; reviewed in Zentralbl. f. Biochem. u. Biophysik, 1912, xiv, 624; ibid, 1913, xv, 913.
(25) Gruber: This Journal, 1913, xxxii, 221 and 438; ibid, 1914, xxxiii, 335.
(26) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(27) Bowditch and Warren: Journ. Physiol., 1886, vii, 416. Bradford: Ibid, 1889, x, 390.
(28) Kaufmann: Arch. de Physiol., 1892, xxiv, 283.
(29) Sherrington: Journ. Physiol., 1909, xxxviii, 382.
(30) Martin: Measurement of induction shocks, New York, 1912.
(31) Cannon and Gruber: This Journal, 1916, xlii, 40.
(32) Tatum: Journ. Pharm. and Exper. Therap., 1912, iv, 155.
(33) Gruber: Loc. cit., 228.
(34) Langley: Proc. Roy. Soc. of London, 1906, lxxviii, 181. Journ. Physiol., 1905, xxxiii, 374–413.
(35) Gruber: This Journal, 1914, xxxiv, 89.
(36) Wilenco: Biochem. Zeitschr., 1912, xlii, 49; Zentralbl. f. Physiol., 1913, xxvi, 1059.

THE EFFECT OF PHOSPHORUS POISONING ON THE CATALASE CONTENT OF THE TISSUES

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In phosphorus poisoning all the organs of the body are injured, the liver being the most affected. This organ first undergoes fatty degeneration with subsequent disintegration of the liver cells. It is supposed that phosphorus causes fatty degeneration by rendering oxidation deficient while the disintegration of the liver cells is supposed to be brought about by the increased autolysis. As a result of the investigations of Jacoby (1), Waldvogel (2), Welsch (3), Aberhalden and Bergell (4), Reiss (5), Wolgemuth (6) and others it is now generally accepted that the essential facts in phosphorus poisoning are an increased rate of autolysis and a defective oxidation in the tissues.

It has been shown in this laboratory that the catalase content is an index to the amount of oxidation in a tissue, being greatest where oxidation is greatest and least where oxidation is least. Furthermore, it was found that when oxidation was decreased in a tissue, as was indicated by a decrease in the catalase content, the tendency of that tissue to undergo autolysis was correspondingly increased. In starvation, for example, it is known that the rate of autolysis is greatly increased in all the tissues of the body except the heart and central nervous system. We found in starving animals that catalase, and hence oxidation, was decreased in those tissues in which autolysis is known to be increased and remained normally high in the heart which is not autolyzed during starvation. Furthermore, in thyroid feeding, it was found that the catalase content, and hence oxidation, was decreased in the fat, skeletal muscles and heart, with a corresponding increase in the rate of autolysis. In view of the fact that the autolyzing enzymes in common with all the ordinary enzymes are easily oxidized and destroyed and that autolysis can be increased in a tissue by decreasing oxidation, and vice versa, the assumption was made that there normally exists a balance between the autolyzing and oxidizing enzymes,

the idea being that when oxidation is increased in a tissue a larger amount of the autolyzing enzymes is oxidized and destroyed with resulting decrease in autolysis, and vice versa.

It is known that autolysis is more intense in the liver than in any of the other organs of animals suffering from phosphorus poisoning. If, according to the preceding hypothesis, the amount of autolysis in an organ is controlled by oxidation, then oxidation should be decreased to a greater extent in the livers of animals suffering from phosphorus poisoning than in any of the other organs. The object of this investigation was to determine if this is true. Cats were used in these experiments. Fifteen of these animals were placed in separate cages. Ten of them were fed daily 60 grams of salmon each, to which 2 mgm. of ordinary yellow phosphorus, previously dissolved in cod-liver oil, had been added; while the remaining five animals were fed the same amount of salmon without the addition of phosphorus. As a rule all of the animals ate the salmon to which the phosphorus had been added, for the first two days. After this some of the animals would eat a part, others all of the material, while some refused to eat any of it.

In the table after "cat fed phosphorus for three days" are given the data from cats that refused to eat the phosphorus after the third day while after "cat fed phosphorus for six days" are given data from animals that had been fed phosphorus for that length of time. When the animals were etherized, approximately 25 cc. of blood were drawn and the blood vessels, by the use of large quantities of 0.9 per cent sodium chloride, were washed free of blood as was indicated by the fact that the wash water gave no test for catalase. The liver and heart were then removed and ground up separately in a hashing machine. Since the blood of the animals that were severely poisoned did not clot it was used without further treatment, while the clotted blood from the less severely poisoned and normal animals was pressed several times through several thicknesses of cheese cloth and ground up in a mortar. The catalase content of the heart was determined by adding 1 gram of the ground material to 45 cc. of hydrogen peroxide, while 1 gram of the liver was added to 500 cc. of hydrogen peroxide in a bottle. A greater amount of hydrogen peroxide was used for the liver because of the greater catalase content of this organ. As the oxygen gas was liberated by the heart muscle, it was conducted through a rubber tube to an inverted burette previously filled with water, and that liberated by the liver to a large, inverted, graduated cylinder. The amount of oxygen gas liberated by the heart and liver

respectively was read off directly from the burette and cylinder where it had displaced the water. After this volume was reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the ground material. In a similar manner the catalase content of the blood was determined by the addition of ten drops of blood to 500 cc. of hydrogen peroxide. A full description of the method may be found in a previous publication (7). It will be

After liver, heart and blood, are given the number of cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 1 gram of the heart, of the liver, and by ten drops of blood, respectively, of normal and phosphorus-poisoned cats

	CAT					AVERAGE AMOUNT OF OXYGEN
	1	2	3	4	5	
						cc.
Liver						
Normal cat	755	620	830	712	610	705
Cat fed phosphorus three days	500	640	602	355	600	539
Cat fed phosphorus six days	250	190	320	340	290	278
Heart						
Normal cat	213	198	266	213	220	222
Cat fed phosphorus three days	165	165	197	179	209	183
Cat fed phosphorus six days	160	162	165	187	140	162
Blood						
Normal	575	640	495	1070	590	674
Cat fed phosphorus three days	602	760	600	720	680	672
Cat fed phosphorus six days	380	420	580	400	630	482

seen that the average amount of oxygen liberated by 1 gram of the liver of the normal animals in ten minutes from 500 cc. of hydrogen peroxide was 705 cc.; that liberated by the liver of cats fed phosphorus three days, 539 cc., and that by cats fed phosphorus six days, 278 cc. of oxygen. The average amount of oxygen liberated by one gram of the heart of the normal cat in ten minutes from 45 cc. of hydrogen peroxide was 222 cc.; that by the heart of cats fed phosphorus for three days 183 cc., and that by cats fed phosphorus six days 162 cc. of oxygen. The average amount of oxygen liberated by ten drops of blood of the normal cats in ten minutes from 500 cc. of hydrogen peroxide was 674 cc.; that by ten drops of blood from the cats fed phosphorus three days, 672 cc., and that by cats fed phosphorus six days, 482 cc. of oxygen.

By comparing the data from the animals that had been fed phosphorus with that from the normal animals it is seen that the catalase content of the livers of the animals that had eaten phosphorus for three days was decreased 23 per cent and 60 per cent in those that had eaten it six days; that the catalase content of the heart was decreased 17 per cent in animals that had eaten phosphorus three days and 27 per cent in those that had eaten it six days; that there was practically no decrease in the catalase content of the blood of the animals that had eaten phosphorus three days while there was 28 per cent decrease in those that had eaten it six days. Since catalase is an index to the amount of oxidation in a tissue, being greatest in amount where oxidation is greatest and least where oxidation is least, the decrease in catalase in the liver, heart and blood of animals fed phosphorus is interpreted to mean that the feeding of phosphorus decreased oxidation in these tissues.

The livers of the animals that had been fed phosphorus three days presented the typical appearance of fatty degeneration with little or no indication of autolysis, while the livers of the cats that had been fed phosphorus six days showed extreme autolysis as well as fatty degeneration. The livers of these severely poisoned animals were literally in a state of falling to pieces as a result of autodigestion. From this it would seem that the amount of autolysis was inversely proportional to the amount of oxidation.

CONCLUSIONS

1. The catalase content of the liver, heart and blood is decreased in phosphorus poisoning, the decrease being greatest in the liver

2. The fact that there was a greater percentage decrease in catalase, and hence in oxidation in the liver, than in the heart, for example, and the fact that autolysis is greater in the liver than in any other organ of the body would seem to lend further support to our contention that oxidation controls the amount of autolysis.

BIBLIOGRAPHY

(1) Jacoby: Zeitschr. f. physiol. Chemie, 1900, xxx, 174.
(2) Waldvogel: Arch. f. klin. Med., 1905, lxxxii, 437.
(3) Welsch: Arch. internat. de Pharm. et de Therap. 1905, xiv, 211.
(4) Abderhalden and Bergell: Zeitschr. f. physiol. Chemie, 1903, xxxix, 464.
(5) Reiss: Berl. klin. Wochenschr., 1905, lxii, 44a, 54.
(6) Wolgemuth: Zeitschr. f. physiol. Chemie, 1905, xliv, 74.
(7) Burge: This Journal, 1916, xli, 153.

THE NATURE AND PROPERTIES OF METATHROMBIN

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The literature concerned with metathrombin presents most widely divergent views as to the composition of the substance and contradictory experiments relating to its behavior. The belief of most later workers is that it is entirely absent from plasma but present in all sera. No one has succeeded in demonstrating the presence of metathrombin in plasma. Fuld (1) expressed the belief that thrombin in solution does not remain unaltered but passes over into an inactive form which on treatment with alkali undergoes hydrolytic cleavage with a new formation of thrombin. Morawitz believed at first that "β proferment" or metathrombin must be produced either by the calcium activation of prothrombin or at the time of fibrin formation, but later he (2) states simply in agreement with Fuld that the greater part of the thrombin formed during coagulation passes over into the inactive form of metathrombin. Weymouth (3) was led to believe that the substance is an antithrombin-thrombin compound. Melanby (4) recognized the existence of an antithrombin-thrombin compound in serum but denied that alkali-activation of serum could split this with the resulting liberation of free thrombin. He records his experiments which led him to the identification of metathrombin with thrombokinase. Collingwood and McMahon (5) support him in this view. During the progress of this work, Gasser (6) has recently offered further evidence in support of Weymouth's theory that metathrombin is an antithrombin-thrombin compound.

These experiments were undertaken at the suggestion of Dr. W. H. Howell to determine something of the composition and significance of metathrombin.

The method of alkali activation used for the detection of metathrombin consisted in the following procedure:

One-half cubic centimeter of the solution to be tested was incubated at room temperature with an equal volume of $\frac{N}{10}$ sodium hydroxide

for fifteen minutes and then neutralized with $\frac{N}{10}$ hydrochloric acid, Neutral Red being used as an indicator. Thrombin liberated by such activation was tested for by the addition of ten drops of a fibrinogen solution to the activated mixture. In all cases a control mixture was made which consisted of 0.5 cc. of the solution to be activated, to which was added ten drops of fibrinogen and a solution of 0.9 per cent sodium chloride, equal in quantity to that of the acid and alkali of the activated specimen. Such a control would reveal any thrombin present before activation.

In solutions in which free thrombin was suspected (in sera, for example), the solution was heated before activation at 60° to destroy this thrombin. If the presence of prothrombin was suspected in the solution to be tested, the solution was first oxalated in order to prevent any further liberation of thrombin from the prothrombin, and then heated to 60°C.

Repeated examinations of fresh and old oxalated plasmas (some of which were heated at 54° to remove the fibrinogen present and thus approach more closely the composition of serum) consistently failed to reveal any trace of metathrombin. It is regularly found in sera, however, as late as two weeks or more after coagulation. There are apparent, then, several possibilities as to the composition and time of formation of metathrombin.

First. Metathrombin might be formed as a result of one of the three main processes concerned in coagulation: (1) the action of the thromboplastic substance; (2) the calcium activation of prothrombin; (3) the combination of thrombin with fibrinogen to form fibrin. As has been stated, the last two possibilities were suggested by Morawitz.

Second. These processes leave in the plasma the following substances: antithrombin, prothrombin, free thrombin, calcium, thromboplastic substance in small amount and fibrin. If the fibrin be removed the remaining substances may be considered as the constituents of serum with which we are interested. In this serum there is present also metathrombin. It is well known that the thrombin of serum suffers a marked diminution within an hour after clot formation and then more gradually disappears, but the metathrombin persists for a much longer time. Pure thrombin, however, may be kept for long periods in solution without losing perceptibly its power of acting on fibrinogen. Alkaline, acid and neutral solutions of thrombin were kept for a week in the presence of calcium without the development of metathrombin. It is difficult to accept the suggestion that metathrombin

is formed by a change of some kind in the thrombin molecule. Experimental work indicates more strongly the occurrence of an interaction of substances. It is probable that the disappearance of thrombin from a serum may merely mark its combination with some other substance of the serum. From this viewpoint, metathrombin might be an antithrombin-thrombin compound, a prothrombin-thrombin compound, a thrombin-calcium compound, or a thrombin-thromboplastic substance compound. That metathrombin is not a prothrombin compound was determined by the alkali-activation of an oxalated serum that had been heated to 60°C. to destroy any free thrombin. Free thrombin was found after activation. Since this solution was calcium-free and thrombin-free to start with, the process of activation must have liberated thrombin rather than prothrombin. If the latter had been liberated it would have remained as prothrombin. It was determined also that alkali activation of isolated prothrombin does not yield free thrombin. The above mentioned possibilities in regard to the origin of metathrombin were each tested experimentally with the following results:

1. That the neutralization of antithrombin by thromboplastic substance is not attended by the formation of metathrombin was determined by incubating oxalated plasma (heated to 54°) with an equal amount of cephalin solution prepared after the method described by Howell (7). Alkali activation of the mixture gave no evidence of the presence of metathrombin. That neutralization of antithrombin by cephalin had taken place in the mixture was determined by antithrombin tests made on the mixture after incubation and at the same time on a control of plasma heated to 54°C., equally diluted with water and incubated for the same period. The cephalin plasma showed a marked decrease in antithrombin content in comparison with the water plasma.

2. The activation of prothrombin by calcium is neither attended by nor necessary for the production of metathrombin. This was determined directly by activating isolated prothrombin with a solution of calcium chloride and testing the thrombic power of the mixture at intervals up to forty-eight hours, carrying parallel tests for metathrombin. The thrombin yield was plentiful and did not decrease perceptibly in the period of the experiment, but at no time was there any indication of the presence of metathrombin.

More positive evidence of the fact that prothrombin activation by calcium is not necessary for the formation of metathrombin was

later obtained in confirmation of results published by Gasser (6) by incubating active thrombin with calcium-free plasma that had been heated to 54°C. and filtered from the fibrinogen precipitate. This mixture on standing developed metathrombin with a corresponding loss of free thrombin as will be described further on.

3. That the actual process of coagulation with the formation of fibrin is not responsible or necessary for the formation of metathrombin was clearly demonstrated by such an experiment as the last mentioned in which metathrombin was developed in a solution in the entire absence of fibrin formation. Further proof that fibrin formation alone cannot cause the production of metathrombin was obtained by clotting pure fibrinogen with pure thrombin; the serum from this coagulation showed no trace of metathrombin. Further, metathrombin was later found to be produced readily by the recalcification of fibrinogen-free plasma, which, of course, is parallel to the experiment of adding thrombin directly to fibrinogen-free plasma, since it has been seen that prothrombin activation plays no part in metathrombin formation.

4. That metathrombin is not a combination of thrombin left uncombined by fibrinogen, with the unactivated prothrombin of serum was determined by adding a solution of calcium-free thrombin to a solution of prothrombin. Activation of this mixture gave no evidence of metathrombin.

5. That the action of calcium on thrombin will not convert it into inactive metathrombin was determined by adding a solution of 0.5 per cent calcium chloride to a solution of pure thrombin. No metathrombin was developed. It was later found quite possible to produce metathrombin by addition of calcium-free thrombin to calcium-free plasma.

6. That metathrombin is not a thrombin-thromboplastic substance combination was determined by the addition of a strong cephalin solution to a pure thrombin solution. Metathrombin was not developed.

7. As this work progressed, the results led steadily to the conclusion that metathrombin is a substance formed by the union of antithrombin and thrombin. Such a union might conceivably take place either after all of the fibrinogen has been satisfied by thrombin, i.e., after a clot has formed, or else the two processes might go on side by side—part of the thrombin liberated from the prothrombin being taken up by fibrinogen to form fibrin and part by the antithrombin present in plasma to form metathrombin, which then becomes detectable in the serum. Later experiments and general considerations of the significance of metathrombin have led to the latter conclusion. The negative re-

sults obtained by experimentally testing other possibilities of the mode of formation of metathrombin served to confirm the theory of Weymouth that metathrombin is an antithrombin-thrombin combination. More direct evidence of the correctness of this theory was obtained by studying the mode of appearance of metathrombin in normal sera as well as by the experimental production of metathrombin in vitro by the recalcification of oxalated plasma and fibrinogen-free oxalated plasma; and finally by the direct addition of thrombin to the equivalent of an antithrombin solution.

The attempt was first made to determine at what point in the process of coagulation metathrombin becomes detectable. It has been the common experience of workers with metathrombin that the serum from normal coagulation apparently contains metathrombin in maximum amounts from the first. Their serum activations seem to have been made between periods of twenty minutes and several hours after coagulation. The attempt was made, therefore, to obtain a centrifugalized serum as soon after clotting as possible in the hope of finding it free from metathrombin. A cat was anesthetized and bled through a cannula from the carotid artery into a clean glass vessel. The blood was whipped with a wire brush to accelerate clotting. As soon as the entire clot had formed, half of the defibrinated serum was immediately oxalated (one part oxalate to eight parts serum). At once the oxalated and unoxalated portions were poured into separate small glass centrifuge tubes which had been packed in the brass centrifuge cups with ice-salt mixture. These cups with their contained tubes had been kept in the freezing mixture during the operation. They were now centrifugalized for seven minutes at high speed and the clear serum was drawn off. Part of the oxalated serum was heated at 60° for one minute to destroy the free thrombin, and alkali activations were made immediately upon the heated oxalated specimen, the unheated oxalated specimen and the unheated unoxalated specimen. Controls for each of these consisted in the same amount of serum diluted with a solution of sodium chloride, 0.7 per cent, in amounts equal to those used in the alkali activation, the same amount of fibrinogen being added in all cases. Activations were then made at intervals, using the unoxalated specimen which was allowed to stand at room temperature. Small portions of this serum were oxalated (one to eight) and heated at 60° one minute just before each test to destroy free thrombin and prevent further calcium activation of the serum prothrombin during alkali activation. Experiments of this type showed that if an activation were made within twelve or thirteen minutes

after clotting, no metathrombin could be detected. The table gives a record of such an experiment.

	ACTIVATED: + FIBRINOGEN	UNACTIVATED: + FIBRINOGEN
1. Serum oxalated immediately after clotting, heated to 60° 13 minutes after clotting	No clot, 24 hours	No clot, 24 hours
2. Serum oxalated and heated 60° 30 minutes after clotting	Membranous clot, 6 hours	No clot, 24 hours
3. Serum oxalated and heated 60° 5½ hours after clotting	Membranous clot, 20 minutes Gel, 40 minutes	No clot, 24 hours
4. Serum oxalated and heated 60° 20 hours after clotting	Firm clot, 11 minutes	No clot, 24 hours
Serum oxalated immediately after clotting		
5. Unheated 13 minutes after clotting	No clot, 7 hours Gel, 24 hours	Gel, 24 hours
6. Unheated 27 minutes after clotting	No clot 1¼ hours Membranous clot, 3 hours	Membranous, 1 hour, 30 minutes
7. Unheated 5½ hours after clotting	Good gel, 5 minutes, 10 seconds	No clot, 1½ hours Clot, 24 hours
8. Unheated 20 hours after clotting	Good clot, 3 minutes, 30 seconds	No clot, 50 minutes Clot, 24 hours
Unheated unoxalated serum		
9. 20 minutes after clotting		Membranous clot, 3 minutes
10. 5½ hours after clotting	Gel, 6 minutes	Gel, 1 hour
11. 20 hours after clotting	Gel, 4 minutes, 20 seconds	No clot, 50 minutes Clot, 24 hours

Experiments of this type show four interesting facts:

1. Metathrombin is not detectable under the conditions of the experiment in cat serum immediately after clotting, (cf. 1 in table).

2. Metathrombin is not formed suddenly in maximum amounts, but grows gradually in the serum. The effect of chilling may be regarded as merely the retarding of the normal process in which metathrombin is possibly formed more rapidly but nevertheless gradually.

3. The thrombin present in large amounts in the serum at first gradually diminishes in amount.

4. The formation of a clot after activation of a serum containing thrombin is no evidence of the presence of metathrombin. It has been stated that activation destroys any free thrombin present. There is no doubt that thrombin is weakened when subjected to alkali-acid activation but that it is by no means destroyed may be seen from a comparison of *1* and *5* in the above experiment. Both of these specimens were oxalated so that no further production of thrombin from prothrombin could be effected; *1* had its thrombin destroyed by heat and did not clot after activation; *5* unheated and containing free thrombin did clot after activation. This latter result must be attributed to the presence of free thrombin in the serum and not to the activation of metathrombin.

The same type of experiment was carried out using oxalated plasma instead of whole blood. Centrifugalized oxalated plasma was recalcified with a determined optimal amount of calcium, whipped, and fibrin removed and a portion immediately oxalated and heated as in the preceding experiment. From this cell-free serum it was possible to remove the fibrin without centrifugalizing and so the serum could be activated as early as three minutes after clotting. Even at this point metathrombin was invariably detectable. A typical experiment will serve:

	ACTIVATED: + FIBRINOGEN	UNACTIVATED: + FIBRINOGEN
Recalcified serum oxalated immediately after clot. Heated 60° 3 minutes after clotting	Membranous clot between 1 and 2 hours	No clot, 24 hours
Recalcified serum oxalated and heated 60° 2½ hours after clotting	Membranous clot, 37 minutes	No clot, 24 hours
Recalcified serum oxalated and heated 60° 20 hours after clotting	Membranous clot, 10 minutes	No clot, 24 hours
Recalcified serum oxalated immediately after clotting, unheated 6 minutes after clotting	Gel, 5½ minutes	Clot, 3 minutes, 15 seconds
Unheated, recalcified serum, oxalated 2½ hours after clotting	Gel, 5 minutes	Membranous, 2 hours
Unheated, recalcified serum, oxalated 20 hours after clotting	Membranous clot, 3½ minutes	No clot, 1 hour Clot, 24 hours
Unheated, unoxalated recalcified serum 7 minutes after clotting	Gel, 4 minutes	Clot, 1 minute, 15 seconds
Unheated, unoxalated recalcified serum 2½ hours after clotting	Gel, 3 minutes 10 seconds	Clot, 6 minutes
Unheated, unoxalated recalcified serum 20 hours after clotting	Membranous, 3 minutes	Membranous, 20 minutes

Metathrombin is then detectable in a serum obtained by recalcification of oxalated plasma earlier than in a serum resulting from normal coagulation. It is well known that an oxalated plasma recalcified with its optimum amount of calcium clots more rapidly than the same plasma allowed to coagulate unoxalated. The effect of recalcification is a more prompt activation of prothrombin with liberation of a relatively larger amount of thrombin in a shorter interval of time than occurs normally. The bearing of such an accelerated thrombin production upon a more speedy metathrombin formation will be seen from other experiments.

A further experiment was carried out with the object of determining whether metathrombin can be detected in unoxalated plasma, and of studying the normal period and mode of appearance of metathrombin. Birds' blood was used in this experiment. A large rooster was anesthetized and bled from the carotid through a paraffined cannula into paraffined tubes which were iced as in the preceding experiments. Part of this plasma was at once centrifugalized, the cell-free normal plasma drawn off and immediately activated, one specimen being heated to 60°C. and one unheated. Neither of these activated unoxalated plasmas or the unactivated controls gave clots during a period of twenty-four hours, indicating that metathrombin cannot be detected by alkali activation in circulating avian plasma.

A second portion of this plasma was not centrifugalized but was allowed to clot while cooled with its cell elements present. Portions of this clotting plasma were taken at intervals from the tube, oxalated, heated to 60° for one minute and activated. The table shows the results.

Plasma taken from carotid of rooster, 10.15 a.m.

10.15 a.m.
0.5 cc. plasma, oxalated, heated 60°, activated, 0.5 cc. fibrinogen added. No clot, 24 hours.

10.35 a.m.
0.5 cc. plasma oxalated, heated 60°, activated, 0.5 cc. fibrinogen added. No clot, 24 hours.

10.50 a.m.
Same procedure.

11.25 a.m.
Fibrin threads present.
Same procedure. Membranous clot, 4 hours.

11.25 a.m.
0.5 cc. plasma oxalated, heated 60°, unactivated, using NaCl 0.9 per cent to make equal dilution. 0.5 cc. fibrinogen added. No clot, 24 hours.

11.35 a.m.

Clot. 0.5 cc. serum, oxalated, heated 60°, activated, 0.5 cc. fibrinogen added. Membranous, 12 minutes. Gel, 15 minutes.

Same unactivated, using NaCl 0.9 per cent to make an equal dilution. No clot, 24 hours.

24 hours later. Same procedure (activating fibrinogen used here one-half as strong as that used above). Gel, 13 minutes.

Same procedure, unactivated, using NaCl 0.9 per cent to make equal dilution. No clot, 24 hours.

Clotting time:

Unoxalated plasma, 1 hour, 20 minutes.

0.5 cc. oxalated plasma, 4 drops 0.5 per cent $CaCl_2$ solution, 30 minutes.

0.5 cc. unoxalated plasma, 4 drops 0.5 per cent $CaCl_2$ solution, 3 hours.

0.5 cc. unoxalated plasma, 4 drops H_2O, 44 minutes.

0.5 cc. unoxalated plasma, 4 drops of Cephalin solution, 6 minutes.

It is seen from such an experiment as this that metathrombin is not detectable in birds' circulating plasma. However, in the slowly clotting blood there is a detectable trace of metathrombin before clot formation is completed. This is suggestive that the processes of metathrombin and fibrin formation go on side by side. It will also be noticed that the recalcified plasma clotted in a much shorter time than the normal plasma.

Experiments such as the three just mentioned demonstrate the fact that metathrombin makes its appearance gradually and its development is attended by a gradual diminution of the previously strong thrombic power of the serum. It is well known that if thrombin be added to serum it is readily inactivated. The possibility suggests itself therefore that this inactivation consists of a union of thrombin with antithrombin and that the gradual disappearance of thrombin from a serum marks the formation of just such a combination, the thrombin of which may be subsequently recovered by alkali activation—in other words, that metathrombin is a union of antithrombin and thrombin formed whenever thrombin is inactivated by antithrombin. If metathrombin is such a combination of antithrombin and thrombin it might be expected that the union would begin to take place in clotting blood by the side of the fibrinogen-thrombin union as soon as thrombin is liberated from prothrombin. The above experiments offer a certain amount of suggestive information in this regard. In cats' plasma allowed to clot unoxalated, and kept at a low temperature, metathrombin was detectable only some minutes after clotting had occurred. In the recalcified plasma under similar conditions it was detectable immediately after clot formation. Here, as stated above, the thrombin was more quickly liberated from prothrombin.

If metathrombin is a compound of antithrombin and thrombin, formed whenever antithrombin inactivates thrombin, it should be possible to produce it experimentally by direct addition of thrombin to antithrombin. A difficulty of this experiment lies in the fact that antithrombin has not been isolated from plasma. It has been shown, however, that there occurs no reaction between thrombin and either thromboplastic substance or prothrombin or fibrinogen which ends in metathrombin formation. The addition of thrombin to an oxalated plasma may be considered then, for experimental purposes, the equivalent of the addition of thrombin to an antithrombin solution, unless one assumes the presence in plasma of some as yet unknown substance with which thrombin combines. Further, it has been seen that coagulation is not necessary for the production of metathrombin. Therefore an oxalated plasma was heated to 54°, the fibrinogen precipitate filtered off and the plasma recalcified. This entailed the liberation of thrombin into the plasma which contained antithrombin. Tests made upon this mixture at first showed an absence of metathrombin and a high thrombin content, which relation after eighteen hours had become relatively reversed. As in the observations upon normal coagulation, there was seen here to be a gradual inactivation of the free thrombin with a corresponding appearance of metathrombin.

Further experimental evidence that the inactivation of thrombin by antithrombin is always attended by the production of metathrombin was obtained by using an oxalated plasma heated at 60° for five minutes. Such treatment destroys the fibrinogen and though the antithrombin is considerably weakened, it is not destroyed. This plasma approximates more closely a free antithrombin solution. With this plasma an experiment was made similar to that described by Gasser (6). Thrombin prepared by Howell's method (8) was dissolved in this plasma and incubated twenty-four hours at 37°. Thrombin and metathrombin tests showed a great decrease in free thrombin accompanied by the appearance of metathrombin. In two such experiments, antithrombin tests were carried out with the object of detecting a decrease in antithrombin content which might be expected if part of the antithrombin is combined by the thrombin. The heating of these plasmas at 60° for five minutes before the addition of thrombin together with the reheating at 60° for two minutes to destroy any free thrombin before each antithrombin test weakened the antithrombin so markedly that the results, while indicating a loss of antithrombin, were not very striking. One such test may be given.

Oxalated plasma heated at 60° for five minutes, incubated with dry thrombin.

0.5 cc. of this thrombin plasma added as soon as made to 0.5 cc. fibrinogen. Firm clot, 1 minute, 30 seconds.

0.5 cc. thrombin plasma 20 hours later, plus 0.5 cc. fibrinogen. No clot, 3 hours.

0.5 cc. thrombin plasma 20 hours later, heated 60° 1 minute, activated, plus 0.5 cc. fibrinogen. Flocculent fibrin, 30 minutes.

0.5 cc. thrombin plasma 20 hours later, heated 60° 1 minute, plus an equivalent amount of 0.7 per cent NaCl plus 0.5 cc. fibrinogen. No clot, 24 hours.

Antithrombin:

1 drop thrombin plasma immediately after mixing, heated to 60° 2 minutes plus 2 drops thrombin (15 minutes) plus 10 drops fibrinogen. Clot, 6 minutes.

Same, with 3 drops thrombin. Clot, 6 minutes.

Same, with 4 drops thrombin. Clot, 6 minutes.

1 drop thrombin plasma 20 hours after mixing, heated 60° 2 minutes plus 2 drops thrombin (15 minutes) plus 10 drops fibrinogen. Clot, 5 minutes.

Same, with 3 drops thrombin. Clot, 2 minutes, 30 seconds.

Same, with 4 drops thrombin. Clot, 2 minutes, 30 seconds.

Metathrombin, then, appears to be produced invariably in solutions that contain both free thrombin and free antithrombin. To test the possibility of its formation in the absence of antithrombin the following experiment was made. All of the known substances concerned in coagulation with the exception of antithrombin were mixed in the following proportions: 4 cc. active prothrombin solution + 4 cc. fibrinogen + 12 drops 0.5 per cent $CaCl_2$ + 20 drops cephalin solution. This mixture clotted and was allowed to stand twenty-four hours before removing the clot. The serum was tested for metathrombin.

0.5 cc. serum immediately after clotting plus 0.5 cc. fibrinogen. Membranous clot, 9 minutes.

0.5 cc. serum 24 hours after clotting plus 0.5 cc. fibrinogen. Membranous clot, 10 minutes.

0.5 cc. serum 48 hours after clotting plus 0.5 cc. fibrinogen. Gel, 8 minutes.

Activation of the serum at these intervals showed no trace of metathrombin. It is noticed that there is no diminution of free thrombin in such a mixture. As far as we know, this coagulation mixture contained every factor concerned in normal coagulation with the exception of antithrombin. It seems reasonable then to assume that the presence of antithrombin is essential for the formation of metathrombin in any solution. Weymouth reached the same conclusion by weakening the antithrombin of oxalated plasma by dialysis and then recalcifying. The serum showed metathrombin in subnormal amounts.

The above experiments indicate the following facts:

1. Metathrombin is not produced in solutions which lack either thrombin or antithrombin.

2. Metathrombin is readily produced in solutions containing both thrombin and antithrombin.

3. In such solutions the free thrombin gradually decreases in amount.

4. There is some evidence that in such solutions the antithrombin also decreases in amount.

It is evident that a combination of thrombin and antithrombin to form metathrombin might provide an efficient method, under certain conditions, for preserving the intravascular fluidity of the blood, as has been suggested recently by Gasser (6).

It was shown by Davis (9) that large amounts of thrombin might be introduced into the circulation without causing thrombosis. Apparently the thrombin in these cases must have been combined with antithrombin and so rendered inactive. In the plasma after such an injection one would expect to find detectable amounts of metathrombin. Accordingly these experiments were repeated with that object in view. It is evident that thrombin may be introduced into the circulating blood either in pure solution or by the indirect method of injecting thromboplastic substance which should cause the activation of prothrombin and the liberation of thrombin in the blood. The latter method was first used.

A cat weighing 2.6 kgm. was anesthetized; the carotid artery of both sides and the femoral vein of the right side were cannulated. The femoral cannula was attached to a burette containing an active solution of cephalin in 0.9 per cent solution of sodium chloride. The activity of this cephalin was determined by its addition to recalcified oxalated plasma immediately before the injection.

From the right carotid cannula 4 cc. of blood were run into a graduate containing 0.5 cc. sodium oxalate, care being taken to make all measurements exact. The graduate was inverted several times to insure thorough mixing of blood and oxalate. Thirty-five cubic centimeters of blood were then removed from the circulation and 15 cc. of cephalin solution were slowly run into the femoral vein from the burette, the entire injection consuming five minutes. Five minutes after the injection, 4 cc. of blood were received from the cannula in the left carotid into 0.5 cc. oxalate as before. The right carotid was then freshly cannulated and half an hour after the injection a third specimen of 4 cc. blood in 0.5 cc. oxalate was obtained. The recannulation of

the left carotid and the withdrawal of a final similar specimen one hour and forty-five minutes after injection, completed the experiment so far as the injection was concerned. The specimens of blood were centrifugalized, the clear plasma drawn off and tested as shown below. It was noted that no perceptible hemolysis had occurred.

Plasma before injection of 15 cc. cephalin = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C
Plasma 1 hour, 45 minutes after injection = D

Metathrombin:

Portions of A, B, C and D were heated to 54° and the fibrinogen precipitate filtered off.

0.5 cc. + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. A + 20 drops 0.9 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. A, unheated, + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

The same series was carried using B, C and D with the same results. After forty-eight hours these activation experiments were repeated. In no case was there detectable a trace of metathrombin.

The antithrombin tests gave the following results:

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	*minutes*
2	A	10	20
3	A	10	15
4	A	10	10
5	A	10	10
2	B	10	25
3	B	10	15
4	B	10	10
5	B	10	10
2	C	10	60
3	C	10	30
4	C	10	25
5	C	10	20
2	D	10	20
3	D	10	15
4	D	10	10
5	D	10	10

A marked rise in antithrombin content is noted, followed by a return to normal. The rise appears to begin shortly after injection. In some instances the five minute specimen exhibited a fall in antithrombin content but in all cases the thirty minute specimen presented a marked rise. The return to normal occurs between thirty minutes and an hour and a half after injection. If the cephalin injected was neutralized by antithrombin we should expect a fall in antithrombin content, and, as stated, this could be detected in some instances in the five minute specimen.

It was believed that the failure to detect metathrombin in these experiments might have been due to a compensatory production of antithrombin which might thus render the cephalin valueless in aiding thrombin production from prothrombin. It has been shown by Delezenne (10), Nolf (11) and others that the liver is probably the seat of antithrombin production. Therefore a similar injection was made in an animal in which both the portal vein and coeliac axis had been ligated, with the object of removing the possibility of any antithrombin output from the liver during the experiment. Exactly the same methods were used in this case as were described in the preceding experiment. There was here a similar marked increase in antithrombin in both the five minute and the thirty minute specimens. Metathrombin was not detected.

Plasma before injection of 15 cc. cephalin = A
Plasma 6 minutes after injection = B
Plasma 26 minutes after injection = C

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP	FIBRINOGEN	CLOTTED
drops		*drops*	*minutes*
2	A	10	35
3	A	10	25
4	A	10	20
2	B	10	45
3	B	10	35
4	B	10	25
2	C	10	1 hour, 30 minutes (imperfect clot)
3	C	10	55
4	C	10	35

Apparently antithrombin was thrown into the circulation from some source other than the liver, for at autopsy the ligatures were found to be intact. It was decided then to establish a head-thorax circulation in an animal with the object of removing the influence of all organs below the diaphragm. A cat was anesthetized, tracheotomy performed and artificial respiration established. The thorax was opened and the aorta and inferior vena cava were ligated just above the diaphragm. Ten cubic centimeters of an active cephalin solution were slowly run into the right external jugular vein. Specimens of blood were collected before and after the injection as in the previous experiments. The antithrombin in this case showed a marked increase in amount in the thirty minute specimen. That the output of antithrombin began very soon after the injection may be inferred from the fact that there was no detectable diminution of antithrombin in the five minute specimen.

Plasma before injection of 10 cc. cephalin = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	*minutes*
2	A	10	20
3	A	10	15
4	A	10	10
5	A	10	10
2	B	10	20
3	B	10	15
4	B	10	10
5	B	10	10
2	C	10	50
3	C	10	35
4	C	10	25
5	C	10	15

Metathrombin was not detected.

That this antithrombin reaction could not take place outside the body was demonstrated by incubating whole oxalated plasma with similar preparations of cephalin at 37°, testing the antithrombic power before and after incubation as in the injection experiments. The addi-

tion of cephalin caused a diminution in antithrombin content which remained constant. There was no subsequent increase in antithrombin whatever.

To determine whether the cephalin itself was the stimulus for this increased output of antithrombin, an injection of 20 cc. normal saline was made into the femoral vein of a cat weighing 2.7 kgm. Fifty cubic centimeters of blood were drawn before this experiment to approximate the conditions of the cephalin experiments.

Plasma before injection 20 cc. normal saline = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	
3	A	10	No clot, 65 minutes
4	A	10	Clot, 50 minutes
3	B	10	Clot, 40 minutes
4	B	10	Clot, 20 minutes
3	C	10	No clot, 65 minutes
4	C	10	Clot, 55 minutes

This blood was of a very high antithrombin content before injection and the sole effect of the saline seems to have been merely a primary decrease in antithrombin power, followed by a return to normal.

Such experiments were unsatisfactory, however, from the standpoint of metathrombin, and it was decided to resort to the injection of pure thrombin. A very strong thrombin solution was prepared and dialyzed against water until the concentration in sodium chloride was about 1 per cent. An experiment was made upon an intact animal in all details similar to the femoral injections of cephalin described above.

Ten cubic centimeters thrombin solution were run into the femoral vein of a cat.

Plasma before injection of thrombin = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C
Plasma 1 hour, 30 minutes after injection = D

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	
2	A	10	No clot, 2 hours
3	A	10	No clot, 2 hours
2	B	10	No clot, 2 hours
3	B	10	No clot, 2 hours
2	C	10	Clot, 2 hours
3	C	10	Clot, 37 minutes
2	D	10	Clot, 1 hour, 10 minutes
3	D	10	Clot, 57 minutes

Metathrombin:

A, B, C, D heated to remove fibrinogen.

0.5 cc. A+ alkali activation +10 drops fibrinogen—no clot, 24 hours.

0.5 cc. A + 20 drops 0.7 per cent NaCl + 10 drops fibrinogen—no clot, 24 hours

Similar tests were made on B, C and D with identical results. No metathrombin was detected. It is to be noted, however, that the thrombin injected must have been inactivated, for the autopsy revealed no thrombi and the oxalated plasmas remained fluid. Heating to 53° gave a good precipitate of fibrinogen in all cases, further indicating that the fibrinogen had not been combined by the thrombin injected. It will also be noted that this blood was of extraordinary antithrombin content, and the effect of the thrombin injection seems to have been merely the lessening of this antithrombin by combination with thrombin.

There remained then the possibility that metathrombin is gotten rid of in the blood as rapidly as it is formed. To test such a possibility, a cat weighing 3 kgm. was used. The total volume of blood was roughly estimated at 150 cc.; one-third of the amount (50 cc.) was taken from the carotid artery, whipped with a wire brush to hasten coagulation, and the serum filtered off through gauze. This serum containing metathrombin was warmed to body temperature and injected back into the body twenty-five minutes after clot formation.

0.5 cc. serum before reinjection + 1 cc. 0.7 per cent NaCl = A

Oxalated plasma 5 minutes after injection of serum = B

Oxalated plasma 30 minutes after injection of serum = C

Metathrombin:

0.5 cc. A (heated 60° 1 minute and filtered) + alkali activation + 0.5 cc. fibrinogen. Found clotted, 1 hour, 15 minutes.

0.5 cc. A (heated 60° 1 minute and filtered) + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. B (heated 54° 1 minute and filtered) + alkali activation + 0.5 cc. fibrinogen. Scant fibrin, ppt. 24 hours.

0.5 cc. B (heated 54° 1 minute and filtered) + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C (heated 54° 1 minute and filtered) + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C (heated 54° 1 minute and filtered) + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

Here then was an explanation of the fact that metathrombin could not be detected after cephalin and thrombin injections. Even if it were formed, it became quickly indetectable either by removal from the circulation or by the action of some factor existing normally in plasma. To exclude the possibility of body absorption of metathrombin from the circulation, a similar experiment was carried out using plasma outside the body.

Fifty minutes after clotting had occurred, centrifugalized serum was oxalated and heated to 60° for one minute to destroy free thrombin and prevent further activation of the serum prothrombin. The following mixtures were then made:

A = 3 cc. whole oxalated blood + 3 cc. serum; incubated 30 minutes at 37°.

B = 3 cc. centrifugalized oxalated plasma + 3 cc. serum incubated 30 minutes at 37°.

C = 3 cc. centrifugalized oxalated plasma heated to 54° and filtered + 3 cc. serum; incubated 30 minutes 37°.

D = 3 cc. 0.9 per cent NaCl + 3 cc. serum; incubated 30 minutes 37°.

Metathrombin tests made upon these mixtures after heating each to 54° and filtering are shown in the following table:

Metathrombin:

0.5 cc. A + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. A + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. B + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. B + 20 drop s0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. D + alkali activation + 0.5 cc. fibrinogen. Firm clot, 3 hours.

0.5 cc. D + 29 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

It is seen that metathrombin apparently disappears when added to blood outside the body. Further the presence of blood elements plays no part in this disappearance of metathrombin, for centrifugalized plasma exhibited the same result as whole blood. The clot formed after activation of D shows that the metathrombin of the serum was not destroyed by mere dilution. It was possible, of course, that plasma in some way destroyed metathrombin, but there is nothing in plasma so far as is known except fibrinogen which is not present also in serum, and it is clear that the fibrinogen-free plasma exerted the same influence upon metathrombin as the other plasmas. A simpler explanation than that of a destruction of metathrombin by plasma suggests itself:

We have reason to believe that in plasma there is a certain amount of free or uncombined antithrombin. In serum, on the contrary, some of the free antithrombin has been removed by combination with cephalin and the remainder is in combination, loose or firm, with thrombin. When the serum is submitted to alkali activation the antithrombin of the metathrombin is destroyed and the liberated thrombin finds but little antithrombin to combine with and may therefore be detected immediately after the activation. When the plasma is submitted to the same process, some of the free antithrombin is removed but enough remains to insure a rapid combination with the thrombin liberated by the activation and this combination occurs so rapidly as to obscure the detection of the thrombin. If this reasoning is correct, it would follow that a plasma-serum mixture might reveal the presence of metathrombin if the plasma were first heated sufficiently to weaken greatly its content of free antithrombin, or if the mixture were submitted to a reactivation by alkali, on the ground that the first activation would weaken the amount of free antithrombin. The following experiments were therefore made.

Fifty minutes after clot formation, the centrifugalized serum was oxalated and heated at 60° for 1¼ minutes.

2 cc. oxalated plasma unheated + 2 cc. serum incubated 37° for 1 hour = A.

2 cc. oxalated plasma heated 56° and filtered + 2 cc. serum incubated 37° for 1 hour = B.

2 cc. oxalated plasma heated 60° 6 minutes and filtered + 2 cc. serum incubated 37° for 1 hour = C.

2 cc. oxalated plasma heated 70° five minutes and filtered + 2 cc. serum incubated 37° for 1 hour = D.

2 cc. 0.9 per cent NaCl + 2 cc. serum incubated 37° for 1 hour = E.

It is observed that the plasmas added to the metathrombin-containing serum were heated to various temperatures up to 70°, the critical temperature of antithrombin. It is known that heating even to 60° weakens antithrombin.

Metathrombin tests were made after each specimen was heated at 54° one minute and filtered.

Metathrombin:

0.5 cc. A + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. A + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. A activated, neutralized, reactivated, + 0.5 fibrinogen. No clot, 24 hours.
0.5 cc. B + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. B + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. B activated, neutralized, reactivated + 0.5 cc. fibrinogen. Membranous clot, 24 hours.
0.5 cc. C + alkali activation + 0.5 cc. fibrinogen. Good gel, 3 hours.
0.5 cc. C + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. D + alkali activation + 0.5 cc. fibrinogen. Good gel, 3 hours.
0.5 cc. D + 29 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. E + alkali activation + 0.5 cc. fibrinogen. Membranous clot, 1 hour. Solid, 3 hours.
0.5 cc. E + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

It is seen that the presence of metathrombin was detected in the mixtures the plasmas of which had been heated at 60° to 70° with a corresponding weakening of their antithrombin. Further, one activation failed to reveal metathrombin in the 56° plasma mixture (B) whereas a second activation of the solution (then weaker in antithrombin from the effects of the first activation and by reason of some of the antithrombin having combined with the thrombin liberated by this activation) showed the presence of metathrombin.

These experiments explain, possibly, the fact that metathrombin has never been demonstrated in plasma, as well as the failure to detect metathrombin in plasma after injections of cephalin, thrombin or metathrombin itself. The demonstration is in agreement with the injection experiments, as in these cases there was invariably a marked rise in the antithrombin content of the blood. The proof that metathrombin may be present in a solution and yet be "masked" by a high antithrombin content of the solution lends support to the view that metathrombin may be formed regularly in circulating blood as a protective mechanism as suggested above. The free antithrombin content of the plasma would render such small amounts indetectable by alkali

activation. At all events it is clear that the failure to demonstrate metathrombin in the plasma by alkali activation cannot be accepted as an argument against such a view.

CONCLUSIONS

1. Metathrombin is a thrombin-antithrombin compound. The following facts may be offered in support of this view:

a. The formation of metathrombin is not dependent directly upon any of the three essential processes of coagulation, the action of thromboplastic substance, the calcium activation of prothrombin or the formation of fibrin.

b. Metathrombin cannot be produced by the interaction of any known substances concerned in coagulation except thrombin and antithrombin.

c. Metathrombin cannot be produced in any solution from which either thrombin or antithrombin is absent.

d. Metathrombin is readily formed in solutions containing both antithrombin and thrombin.

e. In such solutions the thrombin gradually diminishes in amount.

f. There is evidence that in such solutions the antithrombin also diminishes in amount.

2. Metathrombin added to blood inside or outside the body cannot be detected by the method of alkali activation. The explanation offered for this fact is that the thrombin liberated by the activation is rapidly recombined by the free antithrombin of the blood.

3. On the basis of 2, it is suggested that metathrombin may be constantly forming in circulating blood although not detectable by the method of alkali activation; and that this process may serve to protect the blood from the coagulating effect of thrombin liberated within the circulation.

4. The injection of cephalin (tissue extract) into the external jugular vein (cat) causes a marked increase in the antithrombin content of blood kept circulating through the head and thorax only. No increase in antithrombin content occurs in whole blood to which cephalin is added in vitro at body temperature. This is offered as evidence that the abdominal viscera cannot be regarded as the sole source of antithrombin.

It is a great pleasure to me to thank Dr. Howell for his guidance in this work.

BIBLIOGRAPHY

(1) FULD: Centralbl. f. Physiol., 1903, xxvii, 533.
(2) MORAWITZ: Ergebn. d. Physiol., 1905, iv, 367.
(3) WEYMOUTH: This Journal, 1913, xxxii, 266.
(4) MELANBY: Journ. Physiol., 1908, xxxviii, 28.
(5) COLLINGWOOD AND MCMAHON: Journ. Physiol., 1912, xlv, 119.
(6) GASSER: This Journal, 1917, xlii, 378.
(7) HOWELL: This Journal, 1912, xxxi, 1.
(8) HOWELL: This Journal, 1913, xxxii, 264.
(9) DAVIS: This Journal, 1911, xxix, 160.
(10) DELEZENNE: Travaux de Physiol. (Univ. de Montpellier) 1898.
(11) NOLF: Arch. Internat. d. Physiol., 1910, ix, 407.

THE CHANGES IN CLOTTING POWER OF AN OXALATED PLASMA ON STANDING

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Howell[1] has shown that in testing blood to determine the presence of a hemophilic tendency, it is more satisfactory to obtain the clotting time of the oxalated and centrifugalized plasma after recalcification than to depend upon the time of coagulation of the whole blood, since in the latter case small variations in conditions may make large differences in the figures obtained. In connection with this procedure and also as a matter of general interest, it was thought desirable to ascertain to what extent the coagulating property of an oxalated plasma undergoes alteration upon keeping, and the effect upon this property of temperature and of sterile versus non-sterile conditions. The following experiments were made at the suggestion of Dr. W. H. Howell with the object of testing these points.

It is evident that determinations of the clotting time of blood kept over a period must be made upon oxalate or fluoride plasma. In these experiments, therefore, the following method was adopted:

A cannula was introduced into one of the carotid arteries of an anesthetized cat, and the blood allowed to flow into centrifuge tubes containing one part of 1 per cent sodium oxalate for every eight parts of blood. The blood and oxalate were thoroughly mixed and then centrifugalized for twenty minutes. The cell-free plasma was pipetted off and divided into three parts, one of which was kept at 4°C. during the period of the experiment, another at room temperature and the third at 37°. The clotting time of these specimens was determined at intervals over a period of twenty-four hours by the method of recalcification. It is well known that if calcium be added to a plasma kept fluid by oxalate precipitation of its ionizable calcium the plasma will readily coagulate, the rapidity of coagulation being determined by the

[1] Arch. Int. Med., 1914, xiii, 76.

amount of calcium added. There is for every oxalated plasma a certain "optimum amount" of calcium, the addition of which will cause coagulation in the shortest time possible for that plasma under given conditions. If calcium be added in amounts under this optimum, the clotting time is appreciably slower, and the same is true for calcium in amounts above the optimum. In order to eliminate the errors arising from recalcifying with uncertain proportions of calcium, a series was carried for each test consisting of five clotting tubes, each of which contained 0.5 cc. of the specimen to be tested. To these tubes were added respectively three, four, five, six and eight drops of a 0.5 per cent calcium chloride solution.[2] The tube clotting first was assumed to contain the optimum amount of calcium chloride. For the cat, using this method, the optimum amount of calcium chloride ranged between four and six drops, for different bloods. One such experiment upon human blood showed an optimum of four drops. The amount of calcium necessary to exert this optimum effect upon coagulation remained fairly constant during the period of each experiment, the slight variations falling well within the limits of experimental error. It is realized, when one considers the very minute quantities of calcium which affect coagulation, that even variations in the size of the drops due to temperature changes during the period of the experiment may influence the clotting time.

The coagulation time of the recalcified plasma was found to lengthen markedly during a period of twenty-four hours. In most cases, the variations in the coagulation time of plasma kept at room temperature were negligible up to about four hours after the blood was drawn, when a marked lengthening occurred and persisted steadily, with the result that after twenty-four hours the clotting time was three to seven times as long as it was at the beginning of the experiment.

The temperature at which the plasma is kept was found to exert a definite effect upon the clotting time. The plasma kept at 4° exhibited a very much less loss of clotting power than did that kept at room temperature. Plasma kept at 37°, on the other hand, showed a very much greater loss than that kept at room temperature (fig. 1).

It was noticed in all cases that evidences of putrefaction were most prominent in the specimens kept at 37°—the ones which exhibited the most marked lengthening of the coagulation time during twenty-

[2] The solutions of calcium chloride used for this purpose should be prepared from the crystal or hydrated preparations rather than from the granulated or fused form.

four hours. This was suggestive of the possibility that the loss of clotting power might be the result of bacterial action. Accordingly, sterile plasma was collected in the following manner:

The required amount of oxalate was put into small-necked centrifuge tubes which were plugged with cotton and autoclaved. A clean

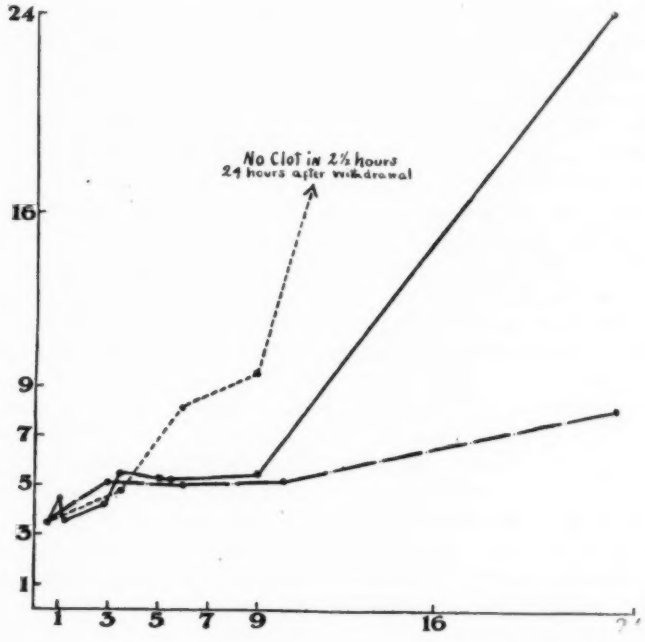

Fig. 1. Showing the loss of clotting power in a non-sterile plasma. One-half cubic centimeter specimens of the plasma were recalcified at intervals with the optimum amount of calcium. Ordinates show the clotting time (in minutes). Abscissae show time lapse (in hours) after withdrawal of blood from artery. Continuous line represents plasma kept at room temperature. Broken line represents plasma kept at 4°C. Dotted line represents plasma kept at 37°C.

operation laid bare the carotid artery of an anesthetized cat. The cotton plug was removed from one of the centrifuge tubes containing the sterile oxalate and a sterile rubber dam was quickly fitted over the mouth. This dam contained a small slit through which one end

of a sterile cannula was plunged, and the other end quickly and carefully inserted into the artery. When the required amount of blood had entered the tube, the cannula was withdrawn and a second sterile rubber dam replaced the one with the slit. The blood was centrifugalized. This method was found of value because it was quite impossible to prevent a cotton plug from being drawn into the tube during centrifugalization. After centrifugalization, the rubber dam was pierced by a long, sterile hypodermic needle and the clear plasma was drawn up into the sterile syringe. Two small flasks had been previously capped with rubber dams and autoclaved. The dam of each of these was now pierced, under sterile precautions, by the hypodermic needle and the plasma was delivered into each. When the needle was withdrawn, the elasticity of the rubber closed the hole to the exclusion of bacteria. Whenever a sample of plasma was needed for clotting time determinations, the dam of the little flask was punctured and the required amount drawn into a sterile syringe and transferred to the clotting tubes. One flask was kept at room temperature and the other at 37°. Plates were made daily from each flask. No colonies were found during the period of the experiment, which lasted five days.

The results of this procedure showed that no change whatever occurred in the clotting time of sterile plasma. The clotting time of the plasma at the end of one hundred and twenty hours was practically identical with the clotting time determined at the beginning of the experiment. This was true for the plasma kept at 37° as well as for that kept at room temperature. The slight variations during the period of the experiment were well within the limits of experimental error. After seventy-two hours, a portion of this sterile plasma was exposed to the air at 37°, and its clotting time at once began to lengthen steadily, so that forty-eight hours after exposure the addition of the optimum amount of calcium caused no coagulation in three hours. The unexposed plasma, however, remained constant in its clotting time (fig. 2).

An experiment was made with the object of determining the cause of the loss of coagulating power in exposed plasmas. Fresh oxalated plasma was heated to 54° and the fibrinogen precipitate filtered off. This fibrinogen-free plasma was then recalcified with the optimum amount of calcium, an active fibrinogen solution was added and the clotting time determined. The same test was made on the fibrinogen-free plasma kept at 37° for forty-eight hours. It is clear that any marked

difference in the clotting times so determined will indicate a change in the prothrombin content of the plasma. Another test made also upon fresh and forty-eight hour plasma consisted in the addition of an active thrombin solution to an unheated plasma. The clotting time of such a mixture will give a relative idea of the condition of the plasma-fibrinogen.

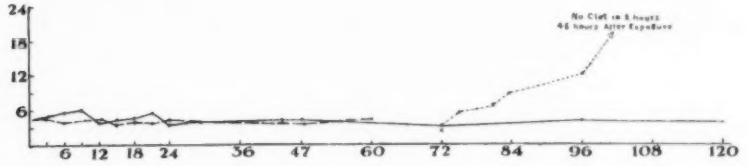

Fig. 2. Showing the retention of clotting power in a sterile plasma. One-half cubic centimeter plasma was recalcified at intervals with the optimum amount of calcium. Ordinates show the clotting time in minutes. Abscissae show time lapse (in hours) after withdrawal of blood from artery. The continuous line represents plasma kept at 37° (sterile). At *X* some of the plasma was exposed to the air at 37°. The change in clotting power of this exposed plasma is followed by the dotted line beginning at *X*. The broken line represents plasma (sterile) kept at room temperature during sixty hours.

The results were as follows:

0.5 cc. fresh oxalate plasma + 5 drops 0.5 per cent $CaCl_2$. Firm clot, 7 minutes, 30 seconds.
0.5 cc. oxalate plasma heated 54° + 5 drops 0.5 per cent $CaCl_2$ + 9 drops fibrinogen. Firm clot, 5 minutes.
0.5 cc. fresh oxalate plasma + thrombin 8 drops. Firm clot, 3 minutes.

Forty-eight hours later

0.5 cc. oxalate plasma + 5 drops 0.5 per cent $CaCl_2$. No clot, 24 hours.
0.5 cc. oxalate plasma heated 54° + 5 drops 0.5 per cent $CaCl_2$ + 9 drops fibrinogen. No clot, 24 hours.
0.5 cc. oxalate plasma + thrombin 8 drops. Poor floating clot, 10 minutes.

It is seen that there was a considerable alteration of fibrinogen during the forty-eight hours and a very marked destruction of prothrombin.

CONCLUSIONS

The coagulation time of non-sterile plasma lengthens steadily during the lapse of time after the blood is drawn. This loss of clotting power

is caused by bacterial action upon the prothrombin and fibrinogen and is not manifest in sterile plasma.

Coagulation determinations made on non-sterile plasma after a lapse of several hours from the time of obtaining the blood do not indicate the true coagulating power of the circulating blood. Low temperatures will greatly lessen the change that takes place, but an accurate test of the true clotting power of the circulating blood after a lapse of time, can be made only upon sterile plasma.

THE DIASTATIC ACTION OF SALIVA IN THE HORSE

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So many conflicting statements appear in the literature concerning the secretion and action of the saliva of the horse that the experiments outlined were undertaken in an effort to determine some of the more fundamental facts.

The following excerpts taken from the observations of various workers on the diastatic power of saliva will serve to illustrate the confusion that exists as to the action of ptyalin in the saliva of solipeds:

R. M. Smith (1) states that in almost all animals the diastatic action of saliva is less than in man with the possible exception of herbivora. He further states that the saliva of the horse will convert crushed raw starch into sugar in one-quarter of an hour. F. Smith (2) holds quite an opposite view declaring that "according to the writer's observations on the horse, saliva has no chemical action on the raw starch of its food." He also declares that it is doubtful if ptyalin exists in the herbivora.

Hofmeister (3) reports that "the mixed saliva of the horse has no effect on raw fiber prepared from hay. Such digestion of raw fiber in the horse occurs only in the small intestine."

According to Ellenberger (4) the secretions of the parotid and the submaxillary glands of the horse can convert starch into sugar but this diastatic action is much stronger in the saliva first secreted after a period of rest. In his later work with Scheunert (5), Ellenberger makes the rather conflicting statements that "the saliva of the horse (einhufer) has merely an insignificant action" while in another paragraph it is stated that "starchy foods chewed and swallowed by a horse with an esophageal fistula were sugar free when first caught but contained sugar after standing two minutes."

Goldschmidt (6) reports that the parotid saliva of the horse does not contain ptyalin but a zymogen which is transformed into ptyalin

during mastication. He believes that bacteria give the impulse for the change. He also finds that during precipitation with alcohol the zymogen is changed into active ptyalin.

Ellenberger and Hofmeister (7) state that "the parotid, submaxillary, sublingual and the buccal glands of the horse, sheep, etc., contain ptyalin which converts starch into sugar" and further that "the parotid is, in all species of animals, the richest in the ferment." In a later publication Ellenberger and Scheunert (5) seem to contradict the latter statement when they say "the action of the mixed saliva of the domestic animals is always more intense than that of the secretion of any one of the salivary glands by itself."

C. Roux (1871) is quoted by Hoppe-Seyler (8) as stating that "ptyalin is not found in the saliva of the horse."

Carlson and Crittenden (9) report their tests for diastase in the saliva of the horse to be negative in that mixed saliva in contact with boiled starch for twenty-four hours gave "practically no solvent action."

Colin (10) reports that the saliva of the horse "has not liquefied starch paste even after twenty-four to twenty-six hours of contact."

In the discussion of the saliva of the horse to be found in various text and reference books opinions of the authors are likewise in opposition. Mathews (11) says that "In the horse mixed saliva is said to have a very powerful diastatic action, whereas saliva collected from the parotid ducts is said to be inactive," while Foster (12) declares that in the horse the "amylolytic powers of either mixed saliva or of any one of its constituent juices are extremely feeble." Disselhorst (13) arranges the following in order of the ptyalin content: man, pig, horse, ox. Incidentally R. M. Smith (1) states that the "saliva of the horse is capable of converting cane sugar into grape sugar."

MATERIALS AND METHODS

In the tests that were made to determine the diastatic property of the saliva of the horse both extracts of the glands and freshly secreted mixed saliva were used as well as the pure secretion of the parotid and of the submaxillary. The difficulty of isolating the secretion of the sublingual prevented any experiments being made with that secretion alone.

Two glycerine extracts were used in the tests; one that has been designated as no. 1 being prepared two years before the tests were made; the other glycerine extract, no. 2, was prepared from fresh

glands, minced and placed in glycerine two weeks before the tests. Owing to bacterial decomposition but few tests were made with a water extract, 5 grams minced gland to 10 cc. of water.

Fresh mixed saliva was secured by either clamping open the jaws of the horse and then sponging or by permitting the animal to chew upon a slightly salted sponge. Isolated secretions were secured by placing a cannula in the duct from the gland from which the secretion was desired. Mixed fresh secretion was secured from different horses; the isolated secretions were taken from but one animal.

The tests charted in the table are merely representative tests; all tests made (exceeding 100) are not shown, although it may be stated that the general result was in every case similar to the test recorded. Constant checks (not shown in the table) were made by the use of human saliva under the same conditions; these were in every case found positive in the conversion of starch solution to reducing sugar. Blank checks (starch only) were also used as controls; these in every case were negative.

The shorter tests, up to four hours, were made by placing the tubes in the water bath at 40°C., in the longer tests the tubes were placed in an incubator at the same temperature.

A detailed discussion of the individual tests would seem to be unnecessary since the table shows the general methods used and the results obtained. Briefly these may be summarized as follows:

Freshly secreted mixed saliva from the horse was added to a 2 per cent solution of boiled cornstarch and incubated. At varying intervals a portion of the mixture was tested for reducing sugar by Fehling's test. In the earlier tests ten minute intervals were the rule; uniformly negative results led to the use of longer intervals for testing. All tests were negative up to four hours; most tests were negative up to six hours. All tests showed the presence of reducing sugar after eight hours, while the controls remained negative.

Tests made upon the isolated secretion of the parotid gave very similar results with apparently slightly accelerated action. However this shortened time was never marked, no more so than the variations found in the mixed saliva from the same horse at different times. Obviously it was not feasible to check the mixed saliva from the same horse simultaneously with its isolated parotid secretion.

Both the old and the recent glycerine extracts of the individual glands, as well as the glycerine extracts of all three glands, were uniformly negative in results in tests up to eighteen hours.

The tests made upon the water extracts were negative up to four hours; positive after six hours. Portions of the same glands were used in making the no. 2 glycerine extracts.

Several writers believe that the salivary glands of the horse secrete a zymogen which is later activated. From such statements the following is taken from Ellenberger and Scheunert (5) as being representative:

> Not all ferments are secreted in the active state by the cell; in many cases the action of chemical agencies is necessary to activate them; for example by the action of dilute acids or alkalies. In some cases even the oxygen of the air or oxygen carriers will convert the inactive proferment into an active condition. . . . . It is said that bacteria are kinase formers (p. 60).
>
> We know very little concerning the activation or the activators of proptyalin; it is possible that a kinase is furnished by other parts of the buccal cavity (cytoblastic tissue) or by the gastric mucosa, or that the activator is found in the air or the food (p. 319).

Attempts to activate the possible ptyalinogen which might have been present, were made with negative results. This was attempted by rendering alkaline and neutralizing; by acidifying and neutralizing; by passing air through the mixture; and by precipitating with alcohol (Lintner method). Activation through the action of bacteria during mastication (Goldschmidt, (6)) or through a possible kinase derived from the buccal mucous membrane (Mathews, (11)) apparently should have occurred during the process of securing the mixed fresh saliva. Carlson and Crittenden (9) state that in horses there is "certainly no activation in the mouth."

Experiments to test the possible action of the saliva of the horse upon cellulose were made with both hay and cotton fiber. Cured hay contains reducing sugar and it was therefore necessary to make the tests quantitative. No increase in sugar was found after twelve to twenty hours contact with either the freshly secreted saliva or the glycerine extracts. No test upon cellulose was made with the isolated secretions.

It seems improbable that the feeble diastase found to be present in the saliva of the horse can be of any importance in the animal's economy. It may be that this is but the lymph diastase as found by Carlson and Ryan (14) in the cat. Schafer and Moore (15) have shown that the salivary glands are not essential to life although Swanson (16) finds that upon extirpation of these glands there follows a modification of the quantity and of the acidity of the gastric juice. Colin

(10) states that when in the horse "the parotid ducts were opened to the exterior, in one month the animal lost one-seventh of its initial weight." This experiment was not repeated in the present series.

Negative results were always found in tests made concerning the action of fresh mixed saliva upon cane sugar, contrary to the findings of R. M. Smith (1).

As check experiments glycerine extract of the pancreas of the horse was found to rapidly convert starch solution into reducing sugar, the tests being positive in one minute.

A detailed description of the method followed in a single test will serve as an illustration of method used in all tests charted. For example in experiment 2 (see table) the procedure was as follows:

Five tubes containing 6 cc. 2 per cent boiled cornstarch were placed in the water bath at 40°C. To each of three tubes 1 cc. mixed fresh saliva of the horse was added. A fourth tube was left blank as a control, while to the fifth 1 cc. human saliva was added. Tests for reducing sugar were made at intervals. In three minutes the human saliva digest showed the presence of reducing sugar. Tests upon the horse saliva digest were negative up to four hours; doubtful trace found at seven hours; positive at eight hours. The blank control containing starch solution only was not tested until a positive reaction occurred in that containing horse saliva. When the control was then tested it was always found negative.

The following table summarizes the results obtained:

Action of fresh secretions on boiled starch

NUMBER	2 PER CENT STARCH	SECRETION USED	TIME TESTED AND RESULT (REDUCING SUGAR)
1	6 cc.	1 cc. MF	½, 1, 2 hours (−); 18 hours (+)
2	6 cc.	1 cc. MF	½, 1, 3, 4 hours (−); 7 (+?); 8 hours (+)
3	6 cc.	1 cc. PF	1, 2, 3 hours (−); 4, 5 (+?); 8 hours (+)
4	6 cc.	1 cc. PF	3, 4, 5 hours (−); 6, 7 (+?); 8 hours (+)
5	6 cc.	1 cc. SMF	1, 2, 3, 4, 5, 6 hours (−); 7 (+?); 8 hours (+)
6	8 cc.	2 cc. each MF and 1 per cent Na_2CO_3	1, 2, 3, 4, 5 hours (−); 8 hours (+)
7	4 cc. (paste)	1 cc. MF	No liquefaction after 2 hours; 18 hours (+)
8	4 cc. (paste)	1 cc. MF	No liquefaction after 2 hours; 18 hours (+)

Action on raw starch and cellulose

NUMBER	2 PER CENT STARCH	SECRETION USED	TIME TESTED AND RESULT (REDUCING SUGAR)
9	1 gm. powd.	2 cc. MF	4 cc. H_2O added; negative after 24 hours
10	2 gm. powd.	2 cc. MF	10 cc. H_2O added; negative after 24 hours
11	1 gm. powd.	1 cc. MF	4 cc. H_2O added; negative after 19 hours
12	Cotton	2 cc. MG 1	Negative after 24 hours
13	1 gm. hay	2 cc. MF	6 cc. H_2O added; no increase in sugar after 20 hours
14	1 gm. hay	2 cc. MG 1	Same as 13
15	1 gm. hay	2 cc. MG 2	Same as 13

Action of glycerine extracts

	2 per cent starch		
16	2 cc.	0.5 cc. PG 1	Negative after 1 hour
17	2 cc.	0.5 cc. SMG 1	Negative after 1 hour
18	2 cc.	0.5 cc. SLG 1	Negative after 1 hour
19	Repeated numbers 16, 17 and 18 with G 2; same results		
20	4 cc.	0.5 cc. PG 1 and SLG 1	Negative after 1 hour
21	4 cc.	0.5 cc. PG 1 and SMG 1	Negative after 1 hour
22	4 cc.	0.5 cc. SLG 1 and SMG 1	Negative after 1 hour
23	4 cc.	0.5 cc. each G 1	Negative after 1 hour
24	6 cc.	1 cc. PG 1 and 0.5 cc. SMG 1 and 0.5 cc. SLG 1	Negative after 15 hours
25	Repeated numbers 20 to 24 with G 2; results negative		
26	1 gm. powd.	2 cc. MG 1	Negative after 20 hours
27	1 gm. powd.	2 cc. MG 2	Negative after 20 hours

Attempts at activation

NUMBER	2 PER CENT STARCH	SECRETION USED	TIME TESTED AND RESULT (REDUCING SUGAR)
28	3 cc.	MG 1*, 2 cc.	Negative after 1 hour (*aerated)
29	3 cc.	2 cc. PG 1*	Negative after 1 hour (*aerated)
30	3 cc.	2 cc. SMG 1*	Negative after 1 hour (*aerated)
31	3 cc.	2 cc. SLG 1*	Negative after 1 hour (*aerated)
32	3 cc.	PG 2, pept.	Negative after 1 hour
33	3 cc.	SMG 2, pept.	Negative after 1 hour
34	3 cc.	SLG 2, pept.	Negative after 1 hour
35	3 cc.	MG 2, pept.	Negative after 1 hour
36	3 cc.	2 cc. PG 2*	Negative after 1 hour (*barely acidulated with 1 per cent HCl, and neutralized with solution KOH)
37	3 cc.	2 cc. MG 2	Negative after 1 hour (*same as 36)
38	4 cc.	2 cc. PG 2*	Negative after 1 hour (*6 cc. PG 2) mixed with 1½ cc. 1 per cent sodium carbonate; at end of 1, 2, 3 hours, neutralized and tested)
39	6 cc.	3 cc. PG 2*	Negative after 2 hours (*exposed to air in beaker for 12 days)
40	4 cc.	2 cc. MG 2*	Negative after 1 hour (*as in 38)
41	4 cc.	2 cc. PG 2*	Negative after 24 hours (*PG 2 mixed with equal amount 1 per cent HCl; neutralized with sodium carbonate at intervals of 1, 2, 4 and 24 hours; then tested)
42	4 cc.	2 cc. MG 2*	Negative after 24 hours *(MG 2 treated same as PG 2, number 41)

Additional experiments

43	5 cc.	2 cc. PW	1, 2, 4 hours (−); 6 hours (+)
44	1 gm. sucrose 4 cc. water	1 cc. MF	Negative after 24 hours
45	3 cc.; 2 per cent starch	0.5 cc. Panc. G.	Positive in one minute

Abbreviations: M, mixed secretion; P, parotid; SM, submaxillary; SL, sublingual; F, fresh or normal; G, glycerine extract; 1, old; and 2, recent extracts; W, water extract; pept., precipitated by alcohol.

SALIVARY SECRETION IN THE HORSE

It may be of interest to note some observations made concerning salivary secretion in the horse during the collection of saliva for the foregoing experiments. The facts noted were mere observations and in no sense quantitative or controlled experiments.

R. M. Smith (1) in speaking of the salivary glands of the horse says:

> Further, these glands are insensible to other stimulants (than mastication) such as salt, acids, etc. brought into contact with the mucous membrane of the mouth.

However, G. Colin (10) makes the statement that:

> The parotids secrete: First, when one puts some food in the buccal cavity while with the aid of a very simple apparatus the slightest movement of the jaws is rendered impossible. . . . . Fourth, they act, sometimes very feebly it is true, under the influence of salt. On the other hand they do not secrete when one forces the animal to masticate tasteless material.

While securing some of the mixed saliva used in the experiments outlined the mouth of the horse was held open by a dental speculum so that mastication was impossible. Nevertheless saliva could still be collected by means of a sponge or gauze; by placing salt on the sponge the rate of secretion was noticeably accelerated. Apparently acceleration also occurred when food was placed before the animal, thus confirming Ellenberger's (4) early observation that "the sight of favorite food will also cause a flow of saliva."

At other times the mixed saliva was secured by permitting the horse to chew upon a sponge which had been slightly salted. Under this procedure the secretion was more abundant than with either of the other methods. It would appear that if the ptyalin is normally activated within the mouth cavity during mastication it should have been so activated during the last mentioned method of securing the saliva.

The mixed saliva thus secured was a thin, translucent, sometimes slightly cloudy, alkaline fluid with a peculiar semi-spicy odor. Ferric chloride tests for potassium sulphocyanide were at all times negative which accords with the statement of Kingzett (17) that its presence in saliva is peculiar to man and with that of Ellenberger and Scheunert (5) that the saliva of the horse contains no potassium sulphocyanide. However, R. M. Smith (1) states that it has been detected in sublingual saliva of the horse although absent from parotid saliva.

SUMMARY AND CONCLUSIONS

1. The saliva of the horse, both the mixed saliva and the isolated secretions of the parotid and of the submaxillary glands, contains a diastase capable of converting starch into sugar. The diastase is extremely feeble, requiring at least five hours for the conversion of boiled starch.

2. The action of the diastase (ptyalin?) was not augmented by aeration, by acidifying or by exposing to the action of weak alkalies.

3. The saliva of the horse is inactive on cellulose and on sucrose.

4. The experiments showed no evidence of the secretion of a zymogen with a subsequent conversion into active ptyalin.

5. Salivary secretion may occur in the horse without mastication by stimulation with chemical substances, with an apparent augmentation through the psychic effect of the sight of food; the greatest flow occurs when the horse is permitted to masticate food material.

6. Potassium sulphocyanide is not found in the saliva of the horse.

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BIBLIOGRAPHY

(1) SMITH: Physiology of the domestic animals, Chicago, 1905.
(2) SMITH: Manual of veterinary physiology, Chicago, 1912,
(3) HOFMEISTER: Arch. f. wiss. u. prakt. Thierhielkunde, 1885, xi, 47.
(4) ELLENBERGER: Physiologie der Haussäugethiere, Berlin, 1890.
(5) ELLENBERGER AND SCHEUNERT: Vergleichende Physiologie der Haussäugetiere, Berlin, 1910, 298 et seq.
(6) GOLDSCHMIDT: Zeitschr. f. Physiol. Chem., 1886, x, 273.
(7) ELLENBERGER AND HOFMEISTER: Arch. f. wiss. u. prakt. Thierheilkunde, 1885, xi, 61.
(8) HOPPE-SEYLER: Physiologische Chemie, Berlin, 1877, 186.
(9) CARLSON AND CRITTENDEN: This Journal, 1910, xxvi, 169.
(10) COLIN: Traite de Physiologie Comparee des Animaux, Paris, 1886.
(11) MATHEWS: Physiological chemistry, New York, 1915, 334.
(12) FOSTER: Text book of physiology, Philadelphia, 1895.
(13) DISSELHORST: Anatomie und Physiologie der grossen Haussäugetiere, Berlin, 1912, 252.
(14) CARLSON AND RYAN: This Journal, 1908, xxii, 1.
(15) SCHAFER AND MOORE: Journ. Physiol., 1896, xiii, 19.
(16) SWANSON: This Journal, 1917, xliii, 211.
(17) KINGZETT: Animal chemistry, London, 1878, 49.

THE RELATION BETWEEN THE THROMBOPLASTIC ACTION OF CEPHALIN AND ITS DEGREE OF UNSATURATION[1]

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Evidence has been presented to show that the thromboplastic action of the tissue juices is a property of the unsaturated phosphatide cephalin (1), (2). A solution of cephalin exhibits its most effective power to accelerate the coagulation of the blood immediately after its isolation from the tissue from which it has been prepared—brain, egg-yolk, liver or heart muscle. Cephalin exposed to the atmosphere or kept in a desiccator over calcium chloride gradually loses its thromboplastic action, and this fact obtains for impure cephalin, designated as "laboratory cephalin" (1) and for cephalin prepared in as pure condition as possible (2). Laboratory cephalin prepared two years ago and another specimen six months old now have absolutely no thromboplastic action although when freshly prepared from the tissue and for some time thereafter they exhibited marked power to hasten the coagulation of the blood. Cephalin is classified among the lipoids as an unsaturated phosphatide for it contains in the fatty acid group of its molecule an unsaturated fatty acid of either the linoleic, linoline or carnubic series—cephalinic acid (3). The object of this investigation was to determine if the gradual loss of the thromboplastic action of cephalin, as indicated by a decline of its power of acceleration of the clotting time, bears any relation to its gradual saturation as indicated by its iodine absorption number.

METHODS

Method of testing thromboplastic activity. The action of cephalin in accelerating the coagulation of the blood was tested in the following

[1] Investigated under the tenure of the Robert M. Girvin Fellowship of this department.

manner: Fresh serum and fresh oxalated plasma were prepared from the same animal. By trial the amount of serum was determined which would cause clotting of a definite amount of plasma within a time convenient for observation. The thromboplastic activity of the solutions of cephalin (1 per cent in all experiments) of different degrees of unsaturation was tested by adding it to the mixture of serum and plasma and noting the acceleration in the clotting time. In all of the experiments the tests on plasma, serum and cephalin were compared with a control of plasma, serum and water. The tests were carried out according to the following example:

Control: Oxalated plasma, 8 drops; water, 3 drops; serum, 3 drops. Clot forms in from 5 minutes to 2 hours, usually the clot is imperfect.

Solution to be tested: Oxalated plasma, 8 drops; cephalin solution, 3 drops; serum, 3 drops. Using freshly prepared cephalin a solid clot forms in 1 minute plus or minus.

Determination of the degree of unsaturation

The degree of unsaturation of all specimens of cephalin tested was determined by the standard Hanus iodine absorption method (4).

Experiments with laboratory cephalin

"Laboratory cephalin" is an impure preparation of cephalin but very active thromboplastically. Its mode of preparation is as follows: A fresh pig's brain obtained from the slaughter house is freed as far as possible from membranes and blood and is then ground to a pulp in a mortar. The pulp is spread as thin as possible on a glass plate and dried in a current of warm air. When thoroughly dry the material is ground to a powder and is extracted for four or five hours with an excess of ether. The ether solution is filtered off in a closed space and is passed through the filter paper until the filtrate comes through clear. The ether filtrate is allowed to evaporate in a current of cool air to a moist residue. The residue is extracted twice with an excess of acetone for fifteen to twenty minutes and then twice with an excess of 95 per cent alcohol. Finally the residue is again treated with acetone, the acetone drained off and the material dried in a desiccator.

The cephalin thus prepared has not been thoroughly freed from other brain constituents, principally lecithin and cholesterin, as these substances are only completely separated from the cephalin by repeated precipitation of the ether solution by alcohol and by acetone.

I had on hand a preparation of laboratory cephalin isolated from the tissue six months previously, and through the kindness of Dr. Howell I was able to secure a specimen two years old. Both of these preparations had been freely exposed to the air since their preparation. The six-months-old specimen was further purified by dissolving it in a little ether, which was accomplished by adding a few drops of water and then agitating violently. The ether solution was centrifugalized and a small amount of insoluble material collected at the bottom of the tube was discarded. The clear ether solution was poured into four times its volume of absolute alcohol and the precipitate (cephalin) was shaken for a short time in the alcohol-ether mixture. The precipitate was collected by centrifugalization and dried in air.

The two-year-old preparation could not be completely dissolved in ether—being much more insoluble than the six-months-old preparation—so its iodine number was determined without any purification. This observation that cephalin loses its property of solution in ether as it ages is observed also when chloroform is used as a solvent.

The iodine number of the six-months-old laboratory cephalin was 37; that of the two-year-old specimen was 42. Both specimens were tested for thromboplastic activity with the following results:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Laboratory cephalin—six months old.....No clot in 1 hour
Laboratory cephalin—two years old.....No clot in 1 hour
Control.....Clotted imperfectly in 6 minutes

Not only had the old cephalin lost its thromboplastic activity but it even retarded the coagulation. This retardation of the coagulation may have been due to an acid reaction present in solutions of old cephalin. Solutions or rather emulsions of fresh cephalin are neutral in reaction. The solutions of both of these preparations were acid to litmus, the two-year-old specimen being more strongly acid to litmus than the six-months-old preparation. Possibly the cephalin had undergone a decomposition of its molecule which had freed a portion of the fatty acid content.

Experiments with Levene's hydrocephalin

Hydrocephalin is cephalin which has been reduced by the action of nascent hydrogen. The substance (5) was prepared by Dr. Levene

and through his courtesy I was able to obtain some of it. At the time of its receipt (January, 1916) it possessed no thromboplastic action and it possesses none now, as is shown in the following experiment:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; hydrocephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Hydrocephalin.....Imperfect floating clot in 1 hour, 30 minutes
Control.....Clotted solid in 4 minutes

The iodine number of the reduced cephalin (hydrocephalin) was 33. In solution it gave a slight acid reaction.

Experiments with cephalin prepared by the method of Levene and West

For the purposes of a previous investigation Doctors Levene and West had kindly supplied me with some cephalin prepared by the method described by the authors (6). The method yields cephalin in a high degree of purity. In the quantitative analysis made by the authors only 90 per cent of the original weight of material was obtained in the hydrolytic products. The authors therefore consider the possibility that the material contained some impurity. This specimen was tested when it was received one year ago and was found to possess marked thromboplastic action as is shown in the following experiment carried out at that time:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Cephalin—Levene and West.....Solid clot in 75 seconds
Control.....Poor clot in 1 hour, 30 minutes

Since then this sample of cephalin has been kept in a corked glass tube and in a desiccator, but not protected from the action of air and light. A one per cent solution of it now gives a slightly acid reaction and has lost its thromboplastic action completely as is shown in the following typical experiment:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Cephalin—Levene and West, one year old
Imperfect floating clot in 1 hour, 30 minutes
Control.....Solid clot in 4 minutes

The iodine number of this preparation is now 42. (The degree of unsaturation was not determined when the specimen was received.)

We note from the above experiments that two specimens of old laboratory cephalin, the iodine number of one being 37 and that of the other being 42, had no thromboplastic action although when freshly isolated from the brain they had a strong action in accelerating the clotting of blood. Further, that a preparation of pure cephalin which one year ago possessed a marked thromboplastic action has no action whatever now, and its iodine number is 42. Finally, a preparation of pure cephalin which had been reduced by the action of nascent hydrogen does not now show any thromboplastic action. Its degree of saturation is indicated by its iodine number which was determined to be 33.

Experiments with freshly prepared cephalin

It was now proposed to prepare pure cephalin and to follow its loss of thromboplastic action and its degree of unsaturation, incident to age and environment, by tests made at regular intervals over a period of time. In order to determine what effect, if any, light and exposure to air may have in bringing about a saturation of the cephalin, a portion of the freshly prepared cephalin was allowed to be continually exposed to the light and air, and another portion was placed in a vacuum desiccator. This desiccator was kept in a dark cabinet and was only exposed to light and air for a minute or two every three or four days in order to withdraw a sample of cephalin. Upon removal of the sample a vacuum was immediately produced again within the desiccator.

Preparation of cephalin. Fresh pigs' brains obtained from the slaughter house are freed as far as possible from membranes and blood and then macerated to a pulp in a mortar. The pulp is spread as thinly as possible on glass plates and dried in a current of warm air. When thoroughly dry the material is ground to a powder and extracted with an excess of ether for twelve hours in a shaking machine. The ether solution, which contains the cephalin is grossly separated from the insoluble portion by first passing the material through a fine sieve and then more completely by centrifugalization. The clear reddish-yellow ether solution is poured off and concentrated by evaporation in a current of cool air. The concentrated ether solution is poured slowly into four times its volume of dry acetone with constant stirring of the white flocculent precipitate which results. The precipitate is

collected by centrifugalization, is partially dried in a current of cool air, is dissolved in enough ether to yield a thin solution and then centrifugalized for an hour at high speed. The ether insoluble mass which is thrown down is discarded. The ether solution is concentrated and poured into four times its volume of absolute alcohol and stirred. The precipitate is collected by centrifugalization, dried, dissolved in ether again and centrifugalized. The supernatant clear ether solution is concentrated and again precipitated with absolute alcohol. This process of dissolving in ether, centrifugalizing and precipitating with absolute alcohol is repeated four times. The last alcoholic precipitate is partially dried in a current of cool air, dissolved in ether and precipitated by four times its volume of dry acetone and shaken in a shaking machine for two hours or more in order to dry the cephalin so that it may be obtained as a loose powder. This final precipitate is collected by centrifugalization and dried in the air. The cephalin thus prepared is a yellowish red mass which pulverizes to a light yellow fine loose powder. One hundred and sixty-five grams of brain tissue yielded eight grams of cephalin.

This cephalin was immediately divided into two portions, one to be kept in a vacuum desiccator protected from the action of light and air and the other to be kept continually exposed to light and air. For convenience both of these portions were subdivided into 10 mgm. and 100 mgm. subdivisions. Every three days or so one of the 10 mgm. portions of exposed cephalin was tested for its thromboplastic activity. At the same time and with the same blood a 10 mgm. portion of the cephalin which had been kept in the vacuum desiccator was tested, both tests being controlled by noting the clotting time of the same mixture of plasma and serum to which no cephalin had been added. Either just before or immediately after the test for thromboplastic activity a determination of the degree of unsaturation of both the exposed and protected cephalin was made, using the 100 mgm. portions.

RESULTS

Dog's oxalated plasma and dog's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops

November 27, 1916

Cephalin—freshly prepared. I. A. no. 79	Solid clot in 49 seconds
Control	Poor clot in 20 minutes

December 1, 1916

Cephalin—from vacuum desiccator. I. A. no. 74
Solid clot in 1 minute, 30 seconds

Cephalin—exposed to air and light. I. A. no. 68
Solid clot in 1 minute, 30 seconds

Control.....Sliding clot in 15 minutes

December 4, 1916

Cephalin—vacuum desiccator. I. A. no. 73.....Solid clot in 56 seconds
Cephalin—exposed. I. A. no. 68.....Solid clot in 58 seconds
Control.....Sliding clot in 11 minutes

December 7, 1916

Cephalin—vacuum desiccator. I. A. no. 72.....Solid clot in 58 seconds
Cephalin—exposed. I. A. no. 68.....Solid clot in 1 minute, 18 seconds
Control.....Sliding clot in 9 minutes

December 11, 1916

Cephalin—vacuum desiccator. I. A. no. 68.....Solid clot in 54 seconds
Cephalin—exposed. I. A. no. 64.....Solid clot in 1 minute, 20 seconds
Control.....Sliding clot in 15 minutes

December 14, 1916

Cephalin—vacuum desiccator. I. A. no. 67.....Solid clot in 41 seconds
Cephalin—exposed. I. A. no. 62.....Firm clot in 1 minute, 42 seconds
Control.....Imperfect clot in 9 minutes

December 17, 1916

Cephalin—vacuum desiccator. I. A. no. 64
Solid clot in 1 minute, 13 seconds

Cephalin—exposed. I. A. no. 57.....Solid clot in 2 minutes, 30 seconds
Control.....Sliding clot in 11 minutes

December 20, 1916

Cephalin—vacuum desiccator. I. A. no. 64.....Solid clot in 1 minute
Cephalin—exposed. I. A. no. 57.....Firm clot in 2 minutes
Control.....Sliding clot in 6 minutes

December 28, 1916

Cephalin—vacuum desiccator. I. A. no. 63.....Solid clot in 3 minutes
Cephalin—exposed. I. A. no. 56.....Imperfect clot in 8 minutes
Control.....Imperfect clot in 10 minutes

From the above experiments it is seen that the cephalin when freshly prepared from the brain had on November 27, 1916, an iodine number of 79 and possessed a very active thromboplastic action, but by exposure to light and air had become saturated to an extent indicated by its iodine number of 56 on December 28, 1916, and that it had lost its thromboplastic action to a marked degree. The material kept in the vacuum desiccator gradually became less unsaturated than it orignally was, but the saturation had progressed more slowly and not to the same extent as in the case of the exposed cephalin. Nor had it lost its thrompboplastic activity to the same degree as the exposed

cephalin. Evidently the amount of air admitted to the desiccator when it was opened for the purpose of withdrawing a sample (and there is the possibility of air leakage into the desiccator to be considered) was sufficient to bring about a certain amount of saturation of the cephalin.

Experiments with cephalin kept in vacuum tubes

Thus having found that storage in a vacuum desiccator—to which air must be admitted at intervals and which is always liable to air leakage—is not sufficient to maintain the original degree of unsaturation or of thromboplastic activity, another series of experiments was carried out with freshly prepared cephalin in which each of the subdivisions of the cephalin used in the tests were placed in small glass tubes, after which a very high vacuum was produced and the tube sealed. The tubes were wrapped in cotton and placed in a dark cabinet. The contents were tested for thromboplastic action and the degree of unsaturation at intervals and the results compared with identical determinations carried out at the same time and on the same blood with similar subdivisions of the same preparation of cephalin which had been kept continually exposed to light and air.

RESULTS

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops

February 16, 1917
Cephalin—vacuum tube. I. A. no. 59..Solid clot in 1 minute, 20 seconds
Cephalin—exposed. I. A. no. 45.....Firm clot in 3 minutes
Control.....Jelly clot in 4 minutes, 30 seconds

February 19, 1917
Cephalin—vacuum tubes. I. A. no. 59..Solid clot in 1 minute, 20 seconds
Cephalin—exposed. I. A. no. 42.....Firm clot in 4 minutes, 30 seconds
Control.....Imperfect clot in 6 minutes

February 23, 1917
Cephalin—vacuum tube. I. A. no. 51.....Solid clot in 2 minutes
Cephalin—exposed. I. A. no. 33.....Sliding clot in 9 minutes
Control.....Sliding clot in 6 minutes

February 26, 1917
Cephalin—vacuum tube. I. A. no. 54...Solid clot in 1 minute, 10 seconds
Cephalin—exposed. I. A. no. 31.....Sliding clot in 5 minutes
Control.....Solid clot in 20 minutes

February 28, 1917

Cephalin—vacuum tube. I. A. no. 51.....Solid clot in 2 minutes
Cephalin—exposed. I. A. no. 31.....Firm clot in 6 minutes, 30 seconds
Control.....Firm clot in 8 minutes

March 2, 1917

Cephalin—vacuum tube. I. A. no. 53...Solid clot in 1 minute, 20 seconds
Cephalin—exposed. I. A. no. 31.....Sliding clot in 4 minutes
Control.....Firm clot in 4 minutes

March 5, 1917

Cephalin—vacuum tube. I. A. no. 54.....Solid clot in 2 minutes
Cephalin—exposed. I. A. no. 30.....Poor clot in 10 minutes
Control.....Firm clot in 12 minutes

April 10, 1917

Cephalin—vacuum tube. I. A. no. 54.....Solid clot in 50 seconds
Cephalin—exposed. I. A. no. 31.....Sliding clot in 4 minutes
Control.....Firm clot in 4 minutes

It is noted in the above experiments that the cephalin sealed in the vacuum tubes had maintained its thromboplastic action and practically the same degree of unsaturation over a period of two months, whereas the cephalin exposed to the light and air for this period had gradually lost its ability to hasten the coagulation of the blood and its iodine number had dropped from 59 to 31. It will be recalled that the cephalin saturated by means of nascent hydrogen—Levene's hydrocephalin—possessed no thromboplastic action and its iodine number was determined to be 33.

It will be noticed that the iodine number of the cephalin kept in the vacuum tubes apparently varies slightly. The variations may be due to a slight variation in the amount of material in the tube, incident to placing the material in the tubes, which was accomplished with a small glass funnel and a camel's hair brush.

Although the cephalin used in the above experiment was prepared by the same method as that used in the previous series of experiments in which the material was kept in the desiccator, there was a marked difference in the iodine numbers of the two preparations. When freshly isolated from the tissue the iodine number of the first preparation was 79 and of the second 59. I assume that in the drying of the moist brain pulp the latter preparation became more saturated than the former. Also it is possible that some decomposition of the brain tissue may have taken place before it was thoroughly dried. One preparation of brain cephalin in which a certain amount of decomposition was known to have occurred gave an iodine number of 48 immediately after isolation from the tissue.

Experiments with cephalin exposed to ultraviolet light

In order to intensify the action of the atmosphere and light, cephalin in 10 mgm. and 100 mgm. portions was placed on small watch crystals and exposed to the action of the ultraviolet light rays from a quartz Cooper-Hewlet light and to the ozone in the surrounding atmosphere produced by the passage of the rays through it. This was done with the hope of bringing about much more quickly the same changes in the cephalin which are produced gradually by the action of ordinary light and air. These experiments were made with a very thromboplastically active preparation of cephalin having an iodine number of 64. Five portions of 10 mgm. each and five portions of 100 mgm. each on small watch glasses were placed on a stand 11 cm. under the Cooper-Hewlet light. After exposure for three hours two 10 mgm. and two 100 mgm. portions were withdrawn and tested for thromboplastic activity and degree of unsaturation. At the end of five and one-half hours two more specimens were withdrawn. The remaining specimen was exposed to the ultraviolet light and ozone for ten hours. The results upon the cephalin thus exposed were compared with similar determinations made upon a portion of the same preparation of cephalin which had not been exposed to the action of the ultraviolet light and ozone.

RESULTS

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
December 13, 1916
Cephalin—not exposed to ultraviolet light. I. A. no. 64. Clotted in 1 minute, 10 seconds
Cephalin—exposed to ultraviolet light 3½ hours. I. A. no. 56. Clotted in 3 minutes
Cephalin—exposed to ultraviolet light 3½ hours. I. A. no. 41. Clotted in 4 minutes
Cephalin—exposed to ultraviolet light 5 hours. I. A. no. 50. Clotted in 3 minutes
Cephalin—exposed to ultraviolet light 5 hours. I. A. no. 49. Clotted in 3 minutes, 30 seconds
Cephalin—exposed to ultraviolet light 10 hours. I. A. no. 44. Not clotted in 1 hour
Control. . . . . Clotted in 5 minutes

The combined action of the ozone and the chemically active ultraviolet light had brought about within ten hours a marked reduction

in the iodine number of the cephalin, together with a corresponding decrease in the thromboplastic action. The sample of cephalin which had been exposed for ten hours retarded the coagulation of the blood and gave a slight acid reaction.

CONCLUSIONS

1. The thromboplastic action of cephalin bears a direct relation to its degree of unsaturation, the greater the degree of unsaturation the greater the degree of thromboplastic activity.

2. Cephalin exhibits most effectively its power to hasten the coagulation of the blood shortly after its isolation from the tissues.

3. Cephalin which has become saturated beyond a certain degree, either by reduction or oxidation, completely loses its thromboplastic activity.

4. Cephalin in solution which has become saturated or partly saturated gives an acid reaction and retards the coagulation of blood.

5. As cephalin becomes saturated it gradually loses its property of solution in ether or chloroform.

BIBLIOGRAPHY

(1) HOWELL: This Journal, 1912, xxxi, 1.
(2) McLEAN: This Journal, 1916, xli, 250.
(3) MACARTHUR: Journ. Amer. Chem. Soc., 1914, xxxvi, 2397.
(4) U. S. DEPT. OF AGRIC.: Bureau of Chem., 1910, Bulletin 107, 136.
(5) LEVENE AND WEST: Journ. Biol. Chem., 1916, xxiv, 52.
(6) LEVENE AND WEST: Journ. Biol. Chem., 1916, xxiv, 43.

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